# Weeds

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# *Weeds*

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Weeds

JOURNAL OF THE WEED SOCIETY OF AMERICA

VOLUME IV JANUARY 1956 NUMBER 1

# Taxonomic and Genetic Studies on Wild Oats[1] (*Avena fatua* L.)

D. R. LINDSAY[2]

## INTRODUCTION

THE MAGNITUDE of the problem created by wild oats (*Avena fatua* L.) is one that warrants intensive study not only from the practical but also from the biological standpoint. A knowledge of the entities or taxa present in the wild oats population is a logical beginning to more extensive phases of investigation. The purpose of this paper is to report taxonomic and genetics studies carried out on the wild oats population of the Prairie Provinces. It is hoped that the information obtained may be of use to other investigators interested in the biology of wild oats.

Wild oats is the most serious weed infesting cereal crops in Western Canada. In the spring cereal areas of Canada's three Prairie Provinces and adjacent regions in the United States, annual losses due to wild oats are enormous. Batho (2) has estimated that in Manitoba alone, yearly losses caused by this weed may reach a few millions of dollars. According to other reliable reports, yields in grain fields infested by wild oats may be reduced to a mere fraction of the yield potential. This weed not only reduces the yield and quality of the grain crop but also costs the farmer additional expense in extra tillage practices and handling costs (11).

Wood (11) stated that wild oats was introduced into Western Canada well before the year 1900 during the period of pioneer settlement. Since that early period, wild oats has progressively increased in abundance and importance as a weed. Recently, Leggett (7) has estimated that wild oats now infests to some degree more than 75 per cent of all the cultivated land in the Prairie Provinces.

In Canada, control of wild oats by cultural methods has met with only limited success. Delayed seeding of the crop, fall tillage, the use of smother crops and crop rotations and other cultural control practices have proved useful in some instances but not in others. Leggett (7) states that no adequate cultural control method has yet been found. Chemical control is still largely in the experimental stage.

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The author will not undertake a lengthy description or discussion of the differences between wild, false wild and cultivated oats. For reviews of the vast literature on this subject, the reader is referred to the work of Aamodt *et al.* (1), Hector (4) and Huskins (5). Wild oats may be easily distinguished from the cultivated by: (1) the presence of an oval scar (sometimes referred to as the "suckermouth") surrounded by a dense pubescence at the base of the grain; (2) the presence of a long, heavy, twisted awn on all florets of the spikelet; and (3) the open or spreading type of panicle. Unlike cultivated oats, the grains of wild oats usually possess the property of delayed germination or dormancy which may last from several weeks to a year or more after harvesting (6).

## METHODS

For the present study, the writer has largely followed a classification of wild and cultivated oats made by Malzew (8). This worker divided the *A. fatua* complex into four subspecies: *fatua* (L.) Thell., *cultiformis* Malz., *septentrionalis* Malz., and *meridionalis* Malz. Each of these subspecies has distinctive morphologic characters. Malzew has also divided each subspecies into four varieties according to certain grain characteristics. Although a further separation of the varieties into subvarieties and forms is possible under the Malzew classification, it is not thought advisable to go beyond the variety status so far as the present study is concerned.

Through the generous co-operation of personnel at seven Experimental Stations in Western Canada the author received a large, representative sample of the wild oats population of that area. These workers chose at random infested grain fields in the vicinity of their respective stations and they systematically collected panicles of single wild oat plants at regular intervals in these fields. Notes were taken on the soil type and drainage conditions of each field and on the kind of crop grown at the time of collection. As far as possible, information on past crops and cultivation practices employed for each of the fields selected was also obtained from the farm operator. Collections from 98 fields, with an average sample of 35 panicles for each field, were forwarded to the author for study. A total of more than 3500 specimens was examined and identified according to the Malzew system.

In 1952 and 1953, selections of seed from single plants were grown to maturity in plots of the Botany and Plant Pathology Laboratory at Ottawa. A total of 83 selected lines was established in 1952–53. Since the plot areas were definitely known to be free of previous infestation by wild oats an excellent opportunity was afforded to observe the behavior of the various selections under more or less uniform growing conditions.

To investigate the possibility that natural hybrids between wild and cultivated oats may constitute a part of the wild oats population in Western Canada, a number of preliminary cytogenetic studies were carried out. Using the criteria of (1), irregular meiotic division

and (2), low count of apparently normal pollen as indicators of hybridity, limited cytogenetic studies were made on several selections of wild oats grown in plots.

Several artificial crosses involving selections of *A. fatua* and *A. sativa* were also made and the progeny was grown on to the $F_2$ generation. Grains of all plants were examined morphologically.

## RESULTS

### *Taxonomic Studies*

So far as could be established in the present study all the wild oats in the Prairie Provinces belong to the species *A. fatua*. Other wild species, however, may eventually be found. Most of the specimens examined could also be placed in the *A. fatua* ssp. *fatua* group. Although a few specimens appeared to fit Malzew's description of spp. *cultiformis*, these constituted only a minor portion of the total number of specimens studied. The significance of ssp. *cultiformis* will be discussed in greater detail later in this report.

The material under investigation also contained all four varieties of *A. fatua* L. ssp. *fatua* (L.) Thell. listed by Malzew: var. *intermedia (Lestib.)* Lej. & Court., var. *vilis* (Wallr.) Hausskn., var. *pilosissima* S.F. Gray and var. *glabrata* Peterm. The grain types of these four varieties are shown in Figure 1. The morphologic characters of the grains used to identify each variety are summarized in Table 1.

*Table 1.* Morphologic characters of grains used in the separation of the four varieties of *Avena fatua* L. ssp. *fatua* (L.) Thell.

| Variety | Length of callus hairs in mm. | Lemma pubescence of the primary floret or grain | Grain color |
|---|---|---|---|
| *intermedia* (Lestib.) Lej. & Court. | 1–2 | Dense | Dark brown to black |
| *vilis* (Wallr.) Hausskn. | 1–2 | Glabrous | Yellow to dark gray |
| *pilosissima* S.F. Gray | 3.5–5 | Dense | Dark brown to black |
| *glabrata* Peterm. | 3.5–5 | Glabrous | Yellow to dark gray |

It will be noted that the length of hairs on the callus is the first morphological character used to separate the varieties. Two varieties have short hairs and two have long. Further separation is now possible on the degree of lemma pubescence and grain color. The varieties *intermedia* and *pilosissima* have densely hairy lemmas and dark brown to blackish grains whereas varieties *vilis* and *glabrata* have glabrous lemmas and yellow to dark gray grains.

It is interesting to note that in the separation of the four varieties of *A. fatua* ssp. *fatua* grain color was not used by Malzew. Emme (3) and Vasconcellos (10) however, regard grain color as highly significant in the taxonomy of oats. During the course of this study it was noted that lemma pubescence was variable in some of the material under investigation. Some specimens of var. *vilis* and var. *glabrata*, instead of having glabrous lemmas, were found to have some lemma pubescence. The grain color of these specimens, however, was either yellow, yellowish-gray or gray. Similarly, some speci-

FIG. 1. Varieties of *A. fatua* L. ssp. *fatua* (L.) Thell. From left to right: var. *vilis* (Wallr.) Hausskn.; var. *intermedia* (Lestib.) Lej. & Court.; var. *glabrata* Peterm.; var. *pilosissima* S.F. Gray.

mens of var. *intermedia* and var. *pilosissima* had almost glabrous lemmas instead of the normal densely pubescent grains. Grains of these varieties were always brown or black in color. It was decided to deviate somewhat from the Malzew system and to place more emphasis on grain color. All plants having brown or blackish grains were placed into the var. *intermedia* and var. *pilosissima* categories regardless of the amount of lemma pubescence present; likewise, all specimens with yellow, yellowish-gray or gray grains were classed as var. *vilis* or var. *glabrata*. The final separation in both groups was then based on the length of the callus hairs.

It should be stated that the degree of grain color depends to some extent on the stage of maturity of the plant. In var. *villis* and var. *glabrata*, plants were found which had either all yellow or all gray grains at maturity. More often, yellow and gray grains were found on the same panicle. In such instances, the gray grains were limited to the more mature portions of the panicle. Other plants were found which had yellow primary grains and gray secondary grains. Because of this intermediate color condition it was often difficult to determine the exact grain color of var. *vilis* and var. *glabrata*. Grain color in var. *intermedia* and var. *pilosissima* was much more uniform in mature plants.

*Population studies*

The percentage of presence of the four varieties of *A. fatua* L. ssp. *fatua* (L.) Thell. in the 7 localities from which collections were

made is shown in Table 2. It will be noted that var. *intermedia* was the most common and var. *glabrata* the rarest. There was considerable variation in the percentage of presence of each variety at the different localities. Excluding the rare var. *glabrata,* populations at Brandon and at Indian Head included relatively equal proportions of the remaining three varieties. At Scott, on the other hand, var. *intermedia* and var. *vilis* were present in almost equal amounts and together they formed over 95 per cent of the total population at that station. Although the sample from Melfort was small, var. *vilis* was the most common variety in the collections received, whereas at Lacombe var. *intermedia* was the preponderant type. The populations at Beaverlodge and Lethbridge were similar in that they showed the same trend in the relative prevalence of all four varieties.

*Table 2.* Percentage of presence of varieties of *A. fatua* L. ssp. *fatua* (L.) Thell. for 7 localities in Western Canada.

| Station | No. of panicles examined | Percentage of the total specimens classed | | | |
|---|---|---|---|---|---|
| | | *intermedia* | *vilis* | *pilosissima* | *glabrata* |
| Brandon | 493 | 33 | 22 | 39 | 6 |
| Indian Head | 799 | 32 | 36 | 30 | 2 |
| Scott | 841 | 49 | 48 | 2 | 1 |
| Melfort | 133 | 27 | 68 | 3 | 2 |
| Lacombe | 549 | 62 | 11 | 23 | 4 |
| Beaverlodge | 533 | 51 | 33 | 13 | 3 |
| Lethbridge | 214 | 49 | 27 | 16 | 8 |
| Total | 3,562 | 44 | 33 | 19 | 3 |

The abundance of each variety in individual fields is shown in Table 3. A variety was considered to be dominant if more than 50 per cent of the samples from a field were classed as that variety. In Table 3 it is seen that varieties *intermedia* and *vilis* are the taxa which were dominant in most of the fields sampled. The var. *glabrata* was never a dominant type in any field. Comparison of these data with Table 2 shows a close agreement with regard to the abundance of varieties at the 7 localities.

*Table 3.* Varietal dominance in individual fields at seven localities in Western Canada.

| Station | No. of fields sampled | No. of fields in which no variety was dominant | No. of fields in which varieties were dominant | | | |
|---|---|---|---|---|---|---|
| | | | *intermedia* | *vilis* | *pilosissima* | *glabrata* |
| Brandon | 16 | 7 | 3 | 2 | 4 | 0 |
| Indian Head | 13 | 5 | 2 | 2 | 4 | 0 |
| Scott | 20 | 3 | 8 | 9 | 0 | 0 |
| Melfort | 9 | 0 | 1 | 8 | 0 | 0 |
| Lacombe | 16 | 2 | 12 | 1 | 1 | 0 |
| Beaverlodge | 17 | 2 | 8 | 5 | 2 | 0 |
| Lethbridge | 7 | 3 | 2 | 1 | 1 | 0 |
| Total | 98 | 22 | 36 | 28 | 12 | 0 |

There appeared to be no distinct correlation between varietal dominance and soil type and other ecological factors. Because of the rather scant information on crops grown in the past and tillage

practices employed, it was impossible to relate these factors to varietal dominance. Although climate and soil factors are undoubtedly important in determining the range of wild oats, there is some indication that cultivation practices may be important in the distribution of wild oat taxa. Examination, carried out in 1952, of fields which had undergone a continuous and uniform crop rotation for several years, disclosed a tendency for a single variety to be dominant. Definite conclusions, however, could not be drawn from the information available.

*Growth Tests*

Thus far in this paper, it has been shown that the wild oats under investigation could be classed as four varieties of *A. fatua* L. ssp. *fatua* (L.) Thell. There was, however, considerable variability in the morphologic characteristics of the grains in the specimens examined. It now remains to observe the behavior of various selections of these varieties grown under more or less uniform conditions.

From 83 selected lines established in 1952–53, it was evident that differences occurred not only between varieties but between selections of a single variety. Although germination took place simultaneously in all lines, the early vegetative growth aspects of some selections differed considerably. Some lines had almost prostrate tillers while others had upright culms as in cultivated oats. These growth differences were more evident during the early part of the growing season.

Later in the season some lines differed in times of heading, flowering and maturation of seed. Some selections were as much as 10 days behind others in these dates. Between some lines there was also variation in panicle shape and size and height of plants. Vegetative growth and life cycle, however, was very uniform in all plants of a single selection.

It was observed that the grains of individual plants of 2 lines were considerably larger than those typical of subspecies *fatua*. These selections also had upright culms and contracted panicles resembling cultivated oats. Although the morphologic characters of the grains were similar to subspecies *fatua* in all other ways, they were identified as subspecies *cultiformis* Malz.

A critical examination of mature plant in all selections showed that individual lines of varieties *intermedia* and *pilosissima* were fairly uniform in grain characteristics. Some variability in lemma pubescence and grain color was observed between individual plants of some selections of var. *vilis* and var. *glabrata*. In a few instances, plants with glabrous lemmas gave rise to plants with a few hairs on the primary grains. Color was also somewhat variable. Some gray-grained plants produced offspring with yellow grains.

Growth tests of these lines have shown that each variety consisted of a number of strains or races. There was some evidence of segregation in some selections, especially in varieties *vilis* and *glabrata*. Taxonomic differences between strains were much more evident in vegetative growth habit than in the morphology of mature grains.

Since some selections differed in their life cycle it can be concluded that these strains also differed physiologically.

*Genetic Studies*

All plant material examined at meiosis showed fairly normal meiotic divisions for all varieties of *A. fatua* ssp. *fatua*. All varieties also showed a high percentage of apparently normal pollen. Since known hybrid plants gave similar results it was impossible to determine whether varieties of *A. fatua* were of hybrid origin.

It is interesting to note, however, the results of the experiments in artificial crossing and morphologic studies of the progeny shown in Table 4. In crosses involving *A. sativa* as one of the parents, only segregates of the wild type were recorded.

It will be seen that in the $F_2$ generation plants emerged whose grains resembled the morphologic types of the four varieties previously described. In the cross *A. fatua pilosissima* × *A. fatua vilis*, for example, all four varieties emerged as segregates in the $F_2$ generation. The implications of these crosses will be discussed later in this paper.

*Table 4.* Varieties of *Avena fatua* arising from inter-varietal crosses.

| Artificial cross | Varieties of *A. fatua* in the $F_1$ generation |
|---|---|
| *A. sativa* × *A. fatua intermedia* | *intermedia, vilis* |
| *A. fatua vilis* × *A. sativa* | only *vilis* |
| *A. fatua pilosissima* × *A. fatua vilis* | *pilosissima, vilis, intermedia, glabrata* |
| *A. fatua pilosissima* × *A. fatua intermedia* | *intermedia, pilosissima* |

## DISCUSSION AND CONCLUSIONS

The present study has pointed out the complexity of the wild oat population in Western Canada. It has been shown that most of the specimens examined can be placed in the *A. fatua* ssp. *fatua* (L.) Thell. group. Subspecies *cultiformis* Malz. is also present but in limited amounts. Because of its reputed lack of dormancy this subspecies is not likely to persist under the climatic conditions of the Prairie Provinces. Although most of the specimens could be classed in four varieties of ssp. *fatua*, morphologic characteristics of these varieties tend to be variable. Growth tests indicated that each variety consists of a number of physiological strains or races which differ considerably in their habits of growth. It is possible that these strains may be separated on morphologic characteristics but such a task would be a difficult one. The culture of such strains would be a useful method of separation.

The wild oats populations at the 7 Experimental Stations have shown a wide variation in the relative prevelance of varieties. It has been shown that populations at Brandon and at Indian Head were somewhat similar and that the collections from Scott and from Melfort were different from those from the 3 stations in Alberta. As has been previously stated there seems to be no clear correlation between

the dominance of some varieties in individual fields and general ecological factors.

The meiotic and pollen studies failed to give definite proof that the varieties of *A. fatua* arise through natural crossing. This is understandable since there is a high degree of gene compatibility between *A. fatua* and *A. sativa*. Artificial crosses, however, showed that certain crosses of *A. fatua* varieties and *A. sativa* may give rise to other varieties of *A. fatua*. As shown in Table 4, a study of grain color in all segregates of the crosses carried out disclosed that parent plants having black or brown grains may give rise to segregates with black, brown, gray or yellow grains. On the other hand, parent plants with gray or yellow grains did not give rise to black- or brown-grained plants in the $F_2$ generation. This principle of grain color inheritance has been observed by other investigators and is reported by Emme (3), who stated that black is epistatic over brown, brown over gray and gray over yellow.

Therefore, if this is so, it would be impossible for varieties *vilis* and *glabrata*, which have gray and yellow grains, to produce varieties *intermedia* and *pilosissima*, which have black or brown grains, in a cross of the former varieties with the yellow grained *A. sativa* or between themselves. If natural crossing is involved in the production of *A. fatua* varieties, both var. *vilis* and var. *glabrata* might occur as segregates of a cross in which either var. *intermedia* or var. *pilosissima* is the wild parent.

Inheritance of callus hair length is another interesting point to be considered in the possible origin of varieties of wild oats. Philp (9) and Emme (3) have shown that short hairs are dominant to long in the $F_1$ generation of a cross involving plants with these grain types. In growth tests of both artificial and natural hybrids it was found that neither var. *pilosissima* nor var. *glabrata*, both of which have long callus hairs, segregated for callus hair length, but always bred true for long hairs. Strains of var. *intermedia* and var. *vilis*, however, occasionally segregated into plants with either long or short callus hairs. These strains, therefore, must exist in either of 2 genotypes, S/S or S/s, where S represents the factor for short hairs on the callus. The varieties *pilosissima* and *glabrata* must be homozygous recessive for this factor and of the genotype s/s. Such a genotype can only be produced from wild parents heterozygous for short hairs, for example, var. *pilosissima* from two strains of *A. fatua* var. *intermedia* of genotype S/s.

Population studies have shown that var. *intermedia* was the most common variety of *A. fatua* found in the panicles collected in the Prairie Provinces. It is probable that this variety gives rise to other varieties of *A. fatua* through natural crossing. It has previously been shown that var. *vilis* and var. *pilosissima* can be produced from crosses involving *A. fatua* var. intermedia with *A. sativa*, or, in the case of var. *pilosissima*, from crosses between strains of var. *intermedia* which are heterozygous for the factor of callus hair length. Theoretically, var. *glabrata* can be produced from crosses of certain

segregates which have gray or yellow grain color and the necessary genotype for callus hair length.

Natural crossing between wild and cultivated oats has been frequently reported in the literature. Aamodt *et al.* (1) believed that natural crossing explained the occurrence of so-called "off-type" or aberrant oats found frequently in seed samples in Western Canada. Huskins (5) was of the opinion that natural crossing between wild and cultivated oats occurred more frequently in Western Canada than in other regions because of the nature of the crops grown and the large wild oat population present.

During the present study, natural hybrids between *A. fatua* and *A. sativa* were found at widely separated localities. No attempt was made to identify hybrids between varieties of *A. fatua*. Natural crossing would assure the continuous gene flow between strains of *A. fatua* and *A. sativa* and the subsequent build-up of new physiological races. Such new races would undergo selection by man and by other environmental factors. Malzew (8) refers to the development of specialized weed "forms" in wild oats whose grains closely resemble the grains of other cereals in size and shape. Moreover, strains with certain physiological characteristics such as early ripening and shattering might eventually become adapted as weeds of early maturing cereal varieties. Strains possessing greater dormancy could become more weedy than others and even occupy certain geographical areas.

This paper has stressed the complex taxonomy of wild oats in Western Canada. Fundamental studies in wild oats might well be designed to utilize pure lines and so avoid the variability found in plants grown from seed taken at random. Workers conducting chemical control experiments should be aware that various physiological strains of wild oats occur and that these may influence the effectiveness of chemical treatment and the time of application.

## SUMMARY

1. A taxonomic study was carried out on 3500 panicles of wild oats collected at 7 different localities in Western Canada.

2. Most of the specimens were identified according to the Malzew system as *Avena fatua* L. ssp. *fatua* (L.) Thell. A few specimens were identified as ssp. *cultiformis* Malz. but these constituted only a very minor portion of the total number of panicles examined.

3. All specimens of ssp. *fatua* were classed into four varieties: var. *intermedia* (Lestib.) Lej. & Court.; var. *vilis* (Wallr.) Hausskn.; var. *pilosissima* S.F. Gray and var. *glabrata* Peterm. Separation of these varieties was based on the length of the callus hairs, lemma pubescence and color of the grains. Since lemma pubescence was variable in some of the specimens examined emphasis was placed on grain color as a character for separation of varieties.

4. Population studies showed that the abundance and importance of each variety differed at each of the 7 localities. In some instances, some individual fields showed a preponderance of only one variety.

Neither population differences nor varietal dominance in fields could be correlated with known ecological factors.

5. Growth tests carried out on 83 selections representing each of the four varieties showed that each variety consisted of a number of physiological races or strains. Individual strains in some cases differed considerably in growth habit and life cycle but individual plants of one strain were uniform in these characteristics.

6. Preliminary genetic studies failed to establish whether varieties of wild oats are of hybrid origin. Artificial crosses, however, disclosed that the segregates of some crosses resembled varieties of *A. fatua* ssp. *fatua*.

7. The role of natural crossing between strains of *A. fatua* and *A. sativa* in the production of new races has been discussed.

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# Influence of Soil Type on Soil Sterilization with Sodium Arsenite[1]

R. E. Frans, C. R. Skogley, and G. H. Ahlgren[2]

In weed control, soil sterilization is that process whereby the soil is altered so that it will no longer sustain the higher forms of plant life. In most common use the term refers to the application of chemicals to the soil surface. The sterile effect obtained is not instantaneous but develops as the chemical is carried down through the soil with water or after mixing the chemical with the soil by tillage. The sterile effect obtained is not permanent as leaching, fixation, or decomposition by microbes reduce the toxic effect with time. The length of time chemicals will remain active as soil sterilants varies. Highly soluble materials will be removed much more rapidly than will relatively insoluble compounds.

Soil sterilization with chemicals is not a new practice. Chemicals, such as sodium arsenite, arsenic trioxide, sodium chlorate, and sodium tetraborate, have been used on a commercial scale for many years.

Many of the studies on soil sterilization with chemicals have been conducted on soils and under climatic conditions unlike those in the Northeastern United States. Several studies on soil sterilization with chemicals, with considerable emphasis on arsenic, have been conducted by investigators in California. Recently Crafts and Buck (2), discussing the herbicidal properties of arsenic trioxide, stated that "arsenic is still the most practical and persistent material for soil sterilization."

Research on soil sterilization with arsenicals indicates that soil types are a determining factor in the amount of material needed. Crafts and Rosenfels (3) have shown that at a given rate of arsenic, toxicity is greatest in a sandy loam and lowest in a clay loam. In a clay loam, arsenic is fixed against leaching in the top few inches of the soil while in a sandy loam the arsenic is readily leached. The term "fixation" refers to the process of rendering soluble arsenic insoluble by contact with soil. Fixation appears to be more rapid in an alkaline or neutral medium and is proportional to the amount of limestone present in the soil. Liming a soil increases the amount of arsenic trioxide ($As_2O_3$) fixed, and the naturally calcareous soils, being fully saturated with calcium, will fix the maximum amount of $As_2O_3$. Other factors reported by Boischot and Hebert (1) as influencing fixation are: the per cent clay in the soil, the quantity of arsenic introduced, the time of contact, the quality and nature of the ions linked to the clay, and the soil pH.

[1]Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, New Brunswick.

[2]Formerly Research Assistant, Research Associate and Research Specialist, respectively, Farm Crops Department, Rutgers University, New Brunswick, New Jersey. Credit is due the American Smelting and Refining Company for partial support of this work. Appreciation is also extended to Richard J. Aldrich for helpful suggestions during the course of these experiments.

In view of the lack of information on soil sterilization under New Jersey conditions, this study was started. The main objective was to evaluate, under Eastern climatic and soil conditions, the use of sodium arsenite as a soil sterilant.

## Materials and Methods

The New Jersey trials were conducted on two soil types, a poorly drained phase of Nixon silt loam and Lincroft loamy sand, an excessively drained soil. Plots, 5 by 10 feet in size, were established on both soil types in the fall of 1950. Sodium arsenite was applied to give the equivalent of 100, 250, 500, 1,000, 2,000, and 4,000 pounds of arsenic trioxide per acre. Applications were made directly to the soil surface which remained undisturbed thereafter. Untreated controls were maintained for comparison. The plots were arranged in a randomized block design with four replications.

Six soil cores, selected at random, were taken from each plot 1, 6, 16, and 30 months after applying the arsenite. The cores, which were four inches in diameter and four inches deep, were thoroughly mixed, and a one-quart sample was taken from the composite from each plot. These samples were taken into the greenhouse where they were uniformly seeded with 15 oat seeds. Thirty days after seeding, the oat plants were cut at the soil surface, allowed to air-dry, and then weighed. The total weights per sample were calculated to an average weight per plant. Since greenhouse conditions varied at the different times of sampling, because of the season of the year, the average weights per plant were recalculated as percentages of the controls.

## Results and Discussion

The average relative weights per oat plant grown on treated and untreated plots of Nixon silt loam as effected by rate and time after treatment are given in Table 1. The L.S.D.'s for sampling dates and for rates of application are included. Similar data for the Lincroft loamy sand may be found in Table 2.

*Table 1.* The effect of sodium arsenite on average weights per plant, expressed as percent of controls, of oats grown for 30 days in Nixon silt loam soil taken from treated plots 1, 6, 16 and 30 months after application.

| Rate pounds per acre | Time of sampling after application (months) | | | | Average all dates[2] |
|---|---|---|---|---|---|
| | 1 | 6 | 16 | 30 | |
| No treatment | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 100 | 67.1 | 43.8 | 71.7 | 82.4 | 66.2 |
| 250 | 36.2 | 33.8 | 50.8 | 45.0 | 41.4 |
| 500 | 39.9 | 15.9 | 32.3 | 25.9 | 28.5 |
| 1,000 | 7.6 | 7.6 | 31.2 | 21.7 | 17.0 |
| 2,000 | 6.4 | 6.8 | 17.7 | 19.0 | 12.5 |
| 4,000 | 0.0 | 4.3 | 17.7 | 14.3 | 9.1 |
| Ave. all rates[1] | 36.7 | 30.3 | 45.9 | 44.0 | |

[1]L.S.D. Average dates of sampling, $p < .05 = 6.3$; $p < .01 = 8.2$
[2]L.S.D. Average rates of application, $p < .05 = 12.1$; $p < .01 = 16.6$

*Table 2.* The effect of sodium arsenite on average weights per plant, expressed as percent of controls, of oats grown for 30 days in Lincroft loamy sand soil taken from treated plots 1, 6, 16 and 30 months after application.

| Rate pounds per acre | Time of sampling after application (months) | | | | Average all dates[2] |
|---|---|---|---|---|---|
| | 1 | 6 | 16 | 30 | |
| No treatment | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 100 | 22.9 | 38.7 | 68.6 | 96.2 | 56.6 |
| 250 | 22.3 | 20.7 | 56.5 | 74.4 | 43.5 |
| 500 | 17.9 | 14.6 | 37.3 | 57.4 | 31.8 |
| 1,000 | 19.6 | 14.0 | 32.0 | 32.8 | 24.6 |
| 2,000 | 12.7 | 13.2 | 28.0 | 16.5 | 17.6 |
| 4,000 | 11.6 | 12.8 | 28.9 | 31.2 | 21.1 |
| Ave. all rates[1] | 29.6 | 30.6 | 50.2 | 58.4 | |

[1]L.S.D. Average dates of sampling, $p < .05 = 5.8$; $p < .01 = 7.4$
[2]L.S.D. Average rates of application, $p < .05 = 6.7$; $p < .01 = 9.2$

Considering first the average effect of the sodium arsenite for the total period, it is apparent that inhibition to the growth of oats increased as the rate increased. This was true on both soil types. For the total period a much greater amount of arsenite was required to obtain the maximum inhibition of oats in the sandy soil than in the silt loam.

Although the effects appear to be somewhat varied between times of sampling, it is apparent that there was only a small reduction of toxicity with passage of time at any of the rates studied on the Nixon silt loam. There was, however, considerable reduction of toxicity in the loamy sand, especially at the lower rates.

The data for time of sampling for the the loamy sand show that one month after treatment there were no real differences between any rate of arsenite used. Six months after treatment the 100-pound rate had been greatly reduced in toxicity, but there were no differences from 250 pounds or more. The toxicity of 250 pounds was greatly reduced at the 16-month interval, but there were no differences from 500 pounds or more. Likewise, the 500-pound rate showed a significant reduction 30 months after the sodium arsenite application. The 1,000-pound rate at this date also gave less inhibition than the 2,000-pound rate.

It is generally believed that the toxicity of arsenical compounds in the soil is reduced mainly by leaching and fixation. Since the silt loam soil in this study contained a higher percentage of clay than did the loamy sand, it seems reasonable to assume that the silt loam fixed a higher per cent of arsenite, which accounted for its initial lower toxicity. Using this same logic, it is apparent that more of the arsenite will remain in a soluble form in the loamy sand and thus be subjected to earlier removal because of leaching.

## SUMMARY

1. Data on a 30-month soil sterilization test with sodium arsenite are reported. Six rates of the arsenite were applied on two different soil types—Lincroft loamy sand and Nixon silt loam. Soil samples

were taken from all plots on both soils at four intervals during the period. Oat plants were grown on these soils and compared with oat plants grown on adjacent, untreated soils.

2. At low rates, arsenic toxicity was reduced more rapidly in the loamy sand than in the silt loam even though the initial toxicity was greater in the coarser textured soil.

3. The sandy soil required more sodium arsenite than the finer textured soil to attain a given degree of sterility over the 30-month period.

4. In the Lincroft loamy sand, 1,000 pounds per acre of $As_2O_3$, as sodium arsenite, were required to give favorable soil sterility, as measured by the growth of oats, for the 2½-year period.

5. In the Nixon silt loam soil, no significant improvement in soil sterility was obtained at the end of the 2½-year period with rates above 500 pounds per acre.

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# Evaluation of Selective Weed Control in Wheat[1]

LOWELL W. RASMUSSEN[2]

## INTRODUCTION

ONE of the cultural practises used to control weeds in wheat fields is the application of 2,4–D sprays. The spray is applied as a post emergent selective after the wheat and the weeds have made some growth. The success of selective weed control in wheat depends upon the relative resistance of the wheat plants and the relative susceptibility of the weed plants to the 2,4–D spray. Major factors affecting successful weed control in wheat fields are the stage of growth of both wheat and weed plants, the quantity and formulation of 2,4–D applied, the solvent or carrier for the 2,4–D, the variety of wheat, and the species of weeds present.

Field plot tests have been used to determine the most effective time of spraying, the necessary quantities of 2,4–D, the volume of spray solution, the formulation of 2,4–D, and the relative resistance and susceptibility of weed species and wheat varieties. The information and conclusions to be drawn from these tests are based upon certain criteria for evaluating the effects of the spray on the weeds and the wheat.

Several methods of evaluation have been used in tests conducted in the wheat section of Washington over the past six years. Certain methods definitely are better than others, and doubtless still better methods will be discovered. It is intended that this paper will furnish information on the presently most desirable methods of evaluation as a guide to further studies and for new workers entering this field of study.

## CRITERIA FOR WEED CONTROL EVALUATION

One viewpoint often taken, and frequently justified, is that a critical or precise evaluation of weed control is not necessary. The weeds are either killed or not, and detailed methods are not needed to determine whether a plant is dead or alive. On the other hand, some weed species are not sufficiently susceptible to selective herbicides to be killed outright. When dealing with such plants, some may be killed, some suppressed more or less, and some not affected. Adequate control may be obtained, however, without completely killing the weeds. In some studies the objective is to make comparisons between similar materials, quantities, and time of application. The differences among treatments may not be large with respect to weed control, but they might be significant in certain other respects. Since evaluation other than dead or living plants is often needed, some method must be used. The objective of the evaluation must be to arrive at a figure which properly describes the weed condition on the plot.

[1]Presented as an invitational paper at the First National Weed Control Conference, Kansas City, Missouri, December 8, 1953.

[2]Contribution from the Dept. of Agronomy, State College of Washington, Pullman, Washington.

*Weed Counts*

Counting weeds by separate species or as total weed plants has been used, but generally has been of questionable value. This method merely gives the number of living plants. It gives no indication of the size or vigor of the weedy plants and these are important characteristics from the standpoint of competitive potential. Weed counts fail to give a reliable picture of the weed condition. A few large weeds may be more serious than many small ones. The method is time consuming and tedious.

*Control Ratings*

An arbitrary numerical scale can be set up over any desirable range to evaluate the extent of weed control. In our work we have used a range in which:

1 = excellent control, trace or no live weeds, distinct evidence of killing.
2 = good control, few live weeds but not considered to offer appreciable competition, good evidence of killing.
3 = fair control, a number of weeds remain alive, may range in size from very suppressed to apparently healthy plants, some evidence of killing.
4 = poor control, most weeds apparently making growth, some suppression of size, little or no evidence of killing.
5 = no control, all weeds normal and healthy.

In using the control rating method of evaluation, it is desirable to have three or more individuals rate the plots. Of course, the treatments are not to be known by the persons making the ratings.

This method is fast and reasonably reproducible. There is a minimum of monotony or mental fatigue, consequently personal bias from one time to another is minimized. The data from this cannot be analyzed statistically, but for many studies that is not necessary. The method should not be used where small differences exist and precise comparisons are desired.

*Weed Coverage and Height*

Weed coverage on plots at a suitable time after treatment has been successfully used as an evaluation of weed-killing effect. In some studies it has also been found desirable to determine weed coverage on plots before treatment. Two methods of estimating weed coverage have been adopted in our studies. One method is used for determining the stand of small weeds before treatment, whereas a different method is needed for the after-treatment evaluation when the weeds may be variable in size.

The special frame shown in Figure 1a has been developed for determining the stand of small weeds before treatment. The inside measurements of the frame are 5 x 40 inches, and the squares are $2\frac{1}{2}$ x $2\frac{1}{2}$ inches. The frame is placed at several locations in a plot, following a predetermined pattern. At each location the number of squares containing weeds is recorded. The total or the average weed-containing squares per plot may be used as a criterion of weed stand.

This is the frequency method used by ecologists. A reasonably reliable coverage value can be assumed by determining the percentage of total squares containing weeds.

$$\text{Percentage cover} = \frac{S_w \times 100}{S_t}$$

$S_w$ = squares containing weeds
$S_t$ = total squares = 32 in each sample location

In a plot of 120 square feet (6 x 20 feet), seven locations are sampled which gives a sampling intensity of 8.1 percent.

The method used for determining weed stand after treatment is a modification of a method described by Braun-Blanquet (1) for ecological studies. The plot coverage is based on estimates of several small areas within the plot. The merit of the small area for estimation is that agreement among different people as to plant coverage is good. Plot coverage based on the estimates of several small areas tends to be reliably reproducible by different persons or by one person at different times. After a brief study and practice period, different workers have consistently estimated the same plots within 5 percent of each other.

For the estimation of weed coverage in wheat, a frame was constructed of light metal (Figure 1b). The inside measurements of the

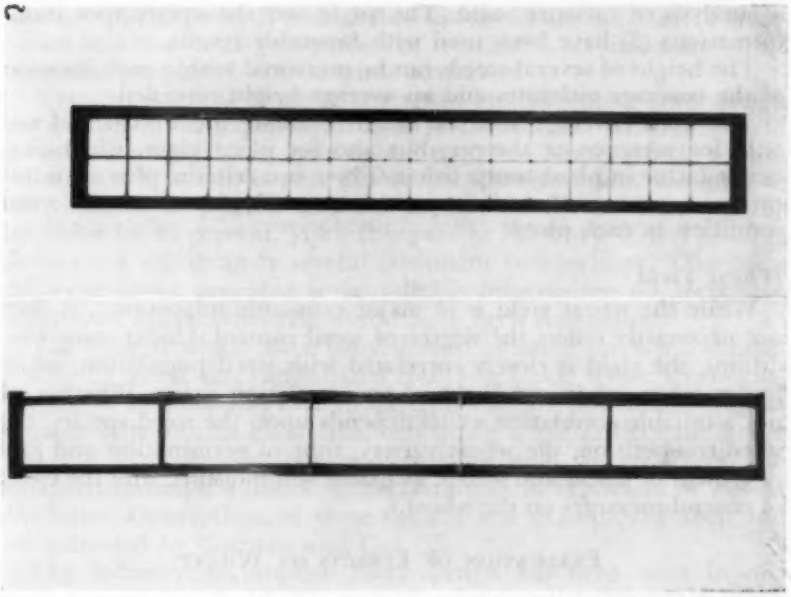

FIG. 1. Frames for estimating stands of weeds. Top—frame for stands of small weeds. Bottom—frame for weeds in grain.

frame are 5 x 60 inches and each frame is divided into 5 sections, each 12 inches long. The frame can be placed between drill rows

of grain and the weed coverage in each 5 x 12 inch section estimated. Broad coverage ranges have been used following a modification of those suggested by Braun-Blanquet (1). The coverage ranges which have proved satisfactory for weeds in wheat are:

| *No weeds* | *0 percent coverage* |
|---|---|
| Trace to 10 percent | 5 per cent coverage |
| Over 10 under 20 percent | 15 percent coverage |
| Over 20 under 40 percent | 30 percent coverage |
| Over 40 under 60 percent | 50 percent coverage |
| Over 60 under 80 percent | 70 percent coverage |
| Over 80 | 100 percent coverage |

Using these ranges makes the decision easy and rapid without sacrificing needed precision. With a large number of estimates, the over and under estimates tend to average out and provide a reliable coverage value for the plot. Within a plot 6 x 20 feet in size, the frame should be placed in at least four locations which gives a 6.9 percent sample. The four locations require 20 estimates to be made in a plot. If it is considered necessary to apply analysis of variance to weed coverage data determined by either of the methods described, it is desirable first to make a frequency distribution of the values. It has been found that the distributions are usually not normal, and a suitable transformation is necessary to make the use of analysis of variance valid. The angle and the square root transformations (3) have been used with favorable results.

The height of several weeds can be measured within each location of the coverage estimates and an average height recorded.

The weed coverage and the height evaluate the weed stand not only for presence or absence, but also for plant vigor, which is a major factor in plant competition. These two criteria, plus a control rating, have been found to provide a good picture of the weed condition in each plot.

*Wheat Yield*

While the wheat yield is of major economic importance, it does not necessarily reflect the degree of weed control. Under some conditions, the yield is closely correlated with weed population, while under other conditions little or no correlation exists. Whether or not a reliable correlation exists depends upon the weed species, the weed competition, the wheat variety, time of germination and rate of growth of wheat and weeds, available soil moisture, and the effect of control measures on the wheat.

## Evaluation of Effects on Wheat

*Plant Measurements*

Plant heights after heading have been used as an indication of treatment effect. The measurement of top and root lengths and their respective weights have been used to determine the effects of 2,4–D on the young wheat plant.

*Yield*

Yield is a principal criterion of herbicidal effect, both because of its economic significance and because it reflects the sum total of many other effects. The measurement of wheat yield both as an indication of weed control and as a criterion of herbicidal effect on the wheat plants raises the question of experimental design and sampling procedures to permit reliable and precise comparisons.

*Experimental Designs*

No single design of experiment is best for all tests and all conditions. Selection of a design to meet the needs of the test in the location is a definite requirement. The value of the results will depend upon how precisely and reliably the comparison can be made. Precision and the making of probability statements regarding treatment differences depend upon the experimental design.

The experimental design need not be complex to provide reliable conclusions from the test; it must, however, be suited to the treatments and the location where the test is to be conducted. In selecting areas and the design to be used, the aim must be to have minimum variation within replicates or blocks. Each treatment must be made on a plot similar to each other plot within the replicate or block. It is often difficult to locate large areas in which both the wheat stand and weed stand are uniform. To avoid serious variation within the replicate area, it is desirable to restrict the number of treatments to a few. If large numbers of treatments are necessary, some type of incomplete block design is advisable.

A typical example of the advantage of an incomplete block design was shown in an experiment with 16 treatments. When the yield data were analyzed as a randomized complete block with five replicates, none of the treatment differences was significant. Removal of the variation associated with the incomplete blocks increased the precision by 44 percent. With this gain in efficiency, it was possible to measure significantly several treatment comparisons. Therefore, the experiment provided some reliable information on treatment differences which otherwise would have been impossible.

The designs which have proved most suitable for weed control studies in wheat are; randomized complete block, where treatments are few in number and four to six replicates are possible; the Latin square, with four to eight treatments; the triple lattice; the 3 x 4 and 4 x 5 rectangular lattices preferably with six replicates; the balanced incomplete block, either arranged in replicates or not in replicates. Descriptions of these designs and examples of their use are provided by Cochran and Cox (2).

The balanced incomplete block design has been used in our studies involving 9, 16, 21, and 25 treatments. With 16 or more treatments there was frequently a worthwhile gain in efficiency. With nine treatments, the value of the incomplete block design is perhaps doubtful, although in some locations it has increased the precision.

*Plot Size and Shape*

It was found that for weed control studies, as well as for the determination of herbicidal effects on wheat, the common 3 row 16-foot-long plot used for varietal testing was not suitable. Studies have not been carried out to determine the best size and shape of plot for selective weed control studies. In fact, there may not be a one best size and shape because of site variations. To facilitate the use of a wheel mounted plot sprayer, plots 6 x 20, 10 x 20 and 12 x 20 feet have been used. These sizes have proved reasonably satisfactory for sampling the weed population. They are not so large, however, as to require excessive area where 60 to 100 plots are needed for a test.

*Sampling for Yield*

Sampling for wheat yield was done by placing an open-sided frame within the plot, avoiding borders, and cutting the wheat plants within the frame. The frame used in our tests is 6 feet long and reaches across three standard drill rows. With 6-inch row spacing the sample taken represents 9 square feet. A sample of 9 square feet from a plot 120 square feet in size constitutes only 7.5 percent of the plot area. In most tests two samples per plot of this size have been taken on which to determine plot yield.

On an experiment conducted at two locations the two samples from each plot were kept separate to permit the calculation of the sampling errors. The coefficients of variation for the two locations were 23.3 and 28.3 while the sampling errors were 16.8 and 13.6 respectively. It was concluded from these data that precision could be improved more by increasing the replications than by taking more samples per plot. In areas where the wheat stand is relatively uniform, one sample per plot might provide a reasonably reliable estimate of plot yield. One of the major difficulties, however, in weed control studies has always been to achieve adequate precision for yield comparisons. Further studies aimed specifically at improving experimental precision seem warranted.

## SUMMARY

Weedy plant species differ in their resistance and susceptibility to herbicidal treatment. Often it is not possible to classify weeds as dead or living after a herbicidal treatment. Therefore, to evaluate properly the effects of herbicides, criteria are needed which reflect the presence or absence of weedy plants together with the vigor and size of growth. Since the principal objection to the presence of weeds is their competitive effect, the evaluation of the weed stand should include some measure of competition potential.

Weed counts are suitable for the determination of the presence of weeds. A broad rating scale has proven suitable for evaluating weed control where small differences are not needed. Plant coverage estimates based on several small areas within a plot provide an indication of presence and size. Coverage estimates as adapted from the ecologists do not fully evaluate competitive potential, however, they provide a closer approach than weed counts. The labor involved in

making coverage estimates is less fatiguing than that for weed counts. While estimates are somewhat subjective, reasonable precision can be attained by a brief training period for workers. The use of several small areas of estimates tends to smooth out individual variations.

Wheat yield is a criterion both of weed control and of direct effects of the herbicide on the wheat plant. The determination of wheat yield requires the use of suitable experimental designs and adequate plot sampling to provide reliable comparisons of yield differences. Experimental designs must be chosen to control as nearly as possible the site and plant stand variations. Four to eight replicates of treatments are recommended. Replicate size should be kept as small as possible to minimize within-replicate variation. If a large number of treatments is necessary, making replicate size large, the use of a design involving incomplete blocks may provide a valuable increase in precision.

Plot size must be kept down to avoid excessive area requirements for the comparison of large numbers of treatments. Plots must be large enough to permit reliable herbicidal application, the sampling of weed population, and an estimate of wheat yield. Plots 6 x 20 feet in size have been used successfully in areas of uniform wheat stands. Under some field conditions, plots 10 x 20 and 12 x 20 feet appear advantageous. Sampling for yield estimations should constitute 5 to 10 percent of the plot area, depending upon the stand variations.

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# Studies on Herbicidal Absorption and Translocation in Velvet Mesquite Seedlings[1]

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THE mesquite tree *(Prosopis* spp.) has been invading southwestern ranges to an alarming degree during the last half century, and has become a serious competitor for available moisture and light with perennial grass—the principal forage of livestock in this area. Its distribution in Arizona, New Mexico, and Texas has been estimated at over 70 million acres. Of the three varieties of mesquite found in these States, velvet mesquite *(P. juliflora* var. *velutina* (Woot.) Sarg.) is essentially limited to Arizona, and is the most common variety found therein (1).

The rate of invasion of this noxious tree has increased considerably since the introduction of livestock, due to the fact that after passage through the digestive tract a large percentage of seeds remains viable. Rodents also play a significant but less important role in the dissemination of seed, as has been described in detail by Reynolds (18) and Reynolds and Glendening (19). Mortality of the seedlings has been studied by Paulsen (17), and quantitative data on the increase of mesquite has been presented by Glendening (12). A comprehensive survey of the mesquite problem in southern Arizona may be found in the recent publication of Parker and Martin (16).

Various methods for killing individual trees are satisfactory, but expensive in dense stands. Burning or cutting down the tree generally results in vigorous sprouting of the dormant buds located just above the root collar (junction of root and trunk). However, burning, when the trees are not over 5 to 10 feet in height, offers definite possibilities of keeping them in check (Humphrey, 14), provided there is sufficient fuel to carry a hot fire. A more positive control which will also kill larger sized individuals may be achieved by basal application of sodium arsenite or diesel oil, as shown by Fisher *et al.* (9) and Roach (21). With this method, kills of 70 percent and better are not uncommon. Studies on herbicidal control by means of injections into the trunk have been made by Blair and Glendening (2). Although effective under certain conditions, this method is not economical for mass application.

Whenever the density of mesquite is such that individual treatment becomes impractical, herbicidal application by airplane appears to be the only answer. To date, optimum results by this method have been achieved with ¾ lb. of a low volatile ester of

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2,4,5–trichlorophenoxyacetic acid (2,4,5–T) per acre applied in an emulsion consisting of 1 gallon of diesel oil and 4 gallons of water. Defoliation has been excellent with this type of application, but final root kill seldom exceeds a mean value of 20 to 25 percent. This mean figure is of slightly less magnitude than Fisher *et al.* (10) have reported for root kills of honey mesquite *(P. juliflora* var. *glandulosa)* in Texas.

The primary goal of present research is to increase the percentage of kill resulting from aerial application of herbicides to mesquite. Since two of the major limiting factors in control of noxious plants appear to be herbicidal absorption and translocation, emphasis was placed on these factors in the study herein described.

## MATERIALS AND METHODS

In order to minimize genetic variation among seedlings, all were progeny of a single parent, and were grown from seed in the greenhouse. Seeds were planted in No. 10 cans which contained vermiculite, and were watered with Hoagland's nutrient solution. Initial experiments had indicated this combination to give maximum and most uniform growth. Greenhouse temperatures were maintained between 30–38°C. during the day and from 20–24°C. at night.

Plants were utilized at an average age of 8 weeks, at which time they consisted of a single stem 20–25 cm. high with 10–14 leaves. Herbicidal application was always made in the afternoon, unless otherwise noted, by painting the upper surface of the designated leaves with a small camel's hair brush. A piece of absorbent paper was held beneath each leaf as it was treated to avoid splashing and contamination of other plant parts. All herbicidal concentrations given are as the acid equivalent, and abbreviations such as 2,4–D, 3,4–D, 2,5–D, and 2,4,5–T refer to the corresponding chlorophenoxyacetic acids. Emulsifying agent concentrations were on a volume-volume basis with respect to the total carrier. Distilled water was used throughout.

## EXPERIMENTAL PROCEDURE AND RESULTS

*Effect of Partial Foliage Treatment and Placement of the Herbicide.* Since it is known that translocation of an herbicide is partly dependent upon the presence of carbohydrate (either endogenous or applied), a preliminary experiment was designed to test the potency of the propyleneglycolbutylether (PGBE) ester of 2,4,5,–T when applied to the upper surface of all leaves of a seedling as compared with application to every third leaflet of each leaf. It was considered that the interstitial untreated leaflets may escape injury for a sufficient time to produce photosynthates which would in turn activate movement of any herbicide that had reached the points of fusion of individual vascular elements leading from the leaflets.

The herbicide consisted of 1,000 ppm. of 2,4,5–T carried in a 1:7 nontoxic oil-water emulsion. One percent of an anionic-nonionic emulsifier was added. In a preliminary experiment eight uniform plants were selected and divided into two sets of four plants each.

On set A, where every third leaflet was treated, 0.3 ml. of herbicide was utilized, whereas set B, with all leaflets treated, used 0.6 ml.

The treated leaflets of all plants were markedly shriveled 24 hours later, whereas the interstitial non-treated leaflets of set A showed no injury for over a week, clearly demonstrating the difficulty with which the herbicide was transported to adjacent leaflets. Apical epinasty, growth, length of stem dieback and ultimate kill were not significantly different (5 percent level) in the two treatments (Table 1). The fact that treatment A utilized only half the amount of herbicide that did treatment B may indicate a greater effect per unit of herbicide when only a portion of the leaves are treated.

*Table 1.* Effect of partial (every 3rd leaflet) and complete leaf treatment upon epinasty, subsequent growth, die back, and percentage kill after application of PGBE ester of 2,4,5–T (1000 ppm.).

| Observations | Every third leaflet treated | All leaflets treated |
|---|---|---|
| Epinasty of apical portion of stem (degrees curvature) | | |
| 2 days after treatment | 85 ± 30°[1] | 28 ± 21° |
| 8 days after treatment | 32 ± 20° | 5 ± 5° |
| Growth during first 15 days following treatment | 1.0 ± 0.1 cm. | 0.4 ± 0.2 cm. |
| Average length of apical portion of stem dead 4 weeks after treatment | 14.8 ± 1.0 cm. | 17.8 ± 1.2 cm. |
| Number of plants dead by 10 weeks following treatment | 2 of 4 | 3 of 4 |

[1]Mean and standard error.

As to herbicidal applications in the field, the above finding would suggest that a given amount of herbicide applied as a coarse droplet spray would be at least as effective as application with a fine mist. Such an application would be in line with the findings of Fisher *et al.* (10). With a coarse droplet spray, not every leaf and leaflet would be contacted, and many would be left to produce photosynthates as previously mentioned. The important advantage of lessened tendency of the larger droplets to drift toward adjacent susceptible crops should also be considered.

In another experiment, 12 plants were selected and divided into 3 groups of 4 each. In one group the 3 youngest leaves of each plant (excluding any young leaf less than 1.0 cm. in length) were treated with 2,500 ppm. of the triethylamine salt of 2,4,5–T. In the second group, 3 centrally located leaves were so treated, and the third group had application made only to the basal 3 leaves of each plant. Carrier for the 2,4,5–T was a 1:4 oil-water emulsion with 1 percent emulsifying agent added. Half the plants of each group were treated with a diesel oil (gravity 38.40) emulsion as carrier, the other half being similarly treated with the same herbicide carried in a nontoxic oil emulsion.

Although initial contact injury resulting from the diesel oil emulsion was greater than with the nontoxic oil, apical epinasty appeared to a much greater extent 1 to 3 days afterwards, with the latter carrier (Figure 1). There was no correlation, however, between type of oil and number of treated leaves having abscissed 4 weeks after treatment.

If both oils are grouped, and the total number of leaves treated at any one position are considered, we find the following number of abscissions 4 weeks after treatment for the 3 positions: apical, 4; mid, 9; basal, 7. Seven weeks after treatment the number of abscissions had increased to 9, 12, and 8, respectively. Regardless of which set of leaves was treated, the youngest non-treated leaves of plants receiving the nontoxic oil carrier showed moderate to severe chlorosis and shriveling 3 to 4 weeks afterwards. The same leaves of plants treated with a diesel oil carrier showed little or no such effect. This preliminary experiment gave the first indication of an enhanced absorption or translocation of 2,4,5–T when carried in the nontoxic oil emulsion described.

*Effect of Pre-dark Treatment and Various Herbicidal Adjuvants.* After a plant has been starved of its native carbohydrates by being placed in darkness or $CO_2$ free air for a time, it is still capable of absorbing and utilizing externally applied sugars, under certain conditions, as demonstrated by Went and Carter (23). The dependency of growth-regulating chemicals upon translocatable carbohydrates for transport from leaf to stem has also been shown by Weintraub and Brown (22). To test the possible ability of glucose and other adjuvants to enhance herbicidal absorption at a time when endogenous carbohydrates were partially depleted, the following experiment was designed.

Sixty-four seedlings were selected for uniformity and half of them were placed in darkness at 08:00 a.m.; the other half remained in the greenhouse. The herbicide was applied at 11:00 a.m. the following day to leaves 4–7 (from the base) of the "dark series" which had just received 27 hours of darkness, as well as to the "light series" taken from the greenhouse. Treatment of the central leaves in this manner gives an indication of translocation to the non-treated distal foliage, as evidenced by formative effect.

Half of each group received 4,000 ppm. of a propyleneglycolbutylether ester formulation of 2,4,5–T and the other half received the same concentration of the sodium salt. All formulations were made up in a 1:4 nontoxic oil-water emulsion, with 2 percent of an anionic emulsifier added in every case.

Each of the four groups was further subdivided by the addition of various adjuvants (4 percent) as follows: (1) glucose, (2) molasses, extracted from *Pinus ponderosa,* and diluted to 4 percent sugar content, of slightly acid pH, (3) latex emulsion, and (4) controls—no further adjuvant.

Of the various observations periodically recorded following treatment, no significant differences were found between any of the adjuvants or between any one adjuvant and the controls. Slight to moderate epinasty of the apical portion of the stem was randomly scattered throughout all treatments involving the sodium salt. Not one plant treated with the ester showed epinasty.

Two days after herbicidal application, leaves treated with the ester were moderately dried and shriveled, whereas those treated with the sodium salt had only a slight mottled bleaching. Nine days

after application, all ester-treated leaves were very severely bleached and shriveled, regardless of pre-treatment. Leaves treated with the sodium salt of 2,4,5–T were variable in appearance. Those on the dark series generally showed less bleaching and shriveling than did those of the light series. In both cases, however, injury was less severe than that resulting from the ester.

Injury to the apical and basal non-treated leaves was just the reverse of the central treated leaves. Thus, of all ester treated plants, 31 percent exhibited mild chlorosis in the apical portion, principally the bud, and none showed any effect in the basal leaves. Among all sodium salt treatments, marked chlorosis was present in both apical and basal foliage of 75 percent of the plants.

Callus formation on the stem was common among the sodium salt-treated plants (Figure 2), but absent from those treated with the ester. On the other hand, nearly all plants eventually sprouted, regardless of pre-treatment or herbicidal formulation. The number of sprouts was tabulated 11 weeks after treatment for all 16 classes according to whether they emerged from nodes apical to, adjacent to, or basal to those of the central treated leaves. The smallest number of sprouts was generally found at the centrally located nodes. A larger number of sprouts, 101, was found emerging from apical and central nodes of all plants treated with the ester as compared to the sodium salt-treated plants which had 23. Correlations of sprout number with other treatments were not evident.

Height measurements (cotyledonary node to apical bud) were taken of all plants during three periods subsequent to treatment, the periods being respectively 2, 2, and 4 weeks. An analysis of variance of growth during individual periods and the combined periods (8 weeks), including all 16 treatments, indicated a significant difference between herbicidal formulations, only, for the final and combined periods. Mean total growth for all ester-treated plants was 2.0 cm., and for sodium salt-treated plants was —0.2 cm.

In general it may be said that the adjuvants used in this experiment resulted in no significant alteration of herbicidal response and that pre-dark treatment had but slight effect. Greatest differences were between formulations of 2,4,5–T. The sodium salt caused less contact injury but greater epinasty, greater injury to distal non-treated foliage and greater repression of growth and sprouting than did the ester.

*Effect of Different Carriers and Emulsifying Agents.* In order to compare a 1:4 diesel oil-water emulsion, a similar nontoxic oil emulsion and distilled water, only, as a carrier for the triethylamine salt of 2,4,5–T (2,500 ppm.) the following experiment was set up. Thirty selected plants were divided into 3 groups of 10 each, and the herbicide was painted on the basal four leaves of all plants. Several emulsifiers (anionic, nonionic, and anionic-nonionic blends) were used within each group at 2 percent strength.

Irrespective of emulsifying agent, all leaves which received the diesel oil-water emulsion were severely shriveled the first day after treatment, whereas the treated leaves of the nontoxic oil-water emul-

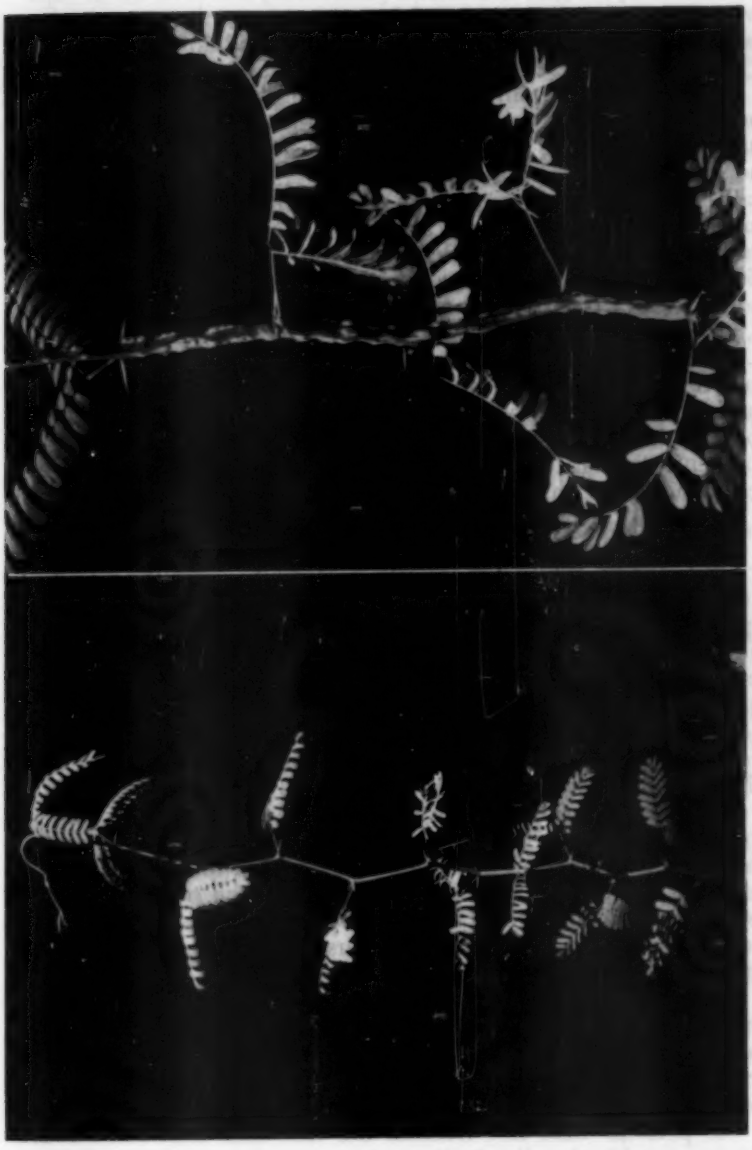


FIG. 1. Terminal epinasty, one week after treatment, resulting from the application of the triethylamine salt of 2,4,5-T in a nontoxic oil-water emulsion to the central three leaves.

FIG. 2. Callus formation, three weeks after treatment, on the stem opposite centrally located leaves treated with the sodium salt of 2,4,5-T in a nontoxic oil-water emulsion.

sion group were moderately shriveled. Herbicide carried in distilled water caused but slight visible foliar injury at this time. On the other hand, the youngest one or two (untreated) leaves of the groups receiving nontoxic oil-water emulsion or water, only, as a carrier consistently showed more extensive chlorosis and formative effect than leaves of the diesel oil group during the first three weeks.

Of the 30 plants, the only ones showing marked callus formation on the stems 15 days after treatment were those which received the formulation containing an anionic emulsifier in conjunction with the nontoxic oil-water emulsion.

If all emulsifiers are grouped, no significant differences in plant kill are noted among the three carriers. At the end of 3 months, 70 percent of the plants were killed with the nontoxic oil emulsion and 60 percent with each of the other two carriers.

Another test of the same nature was next performed, again utilizing 2,500 ppm. of the triethylamine salt of 2,4,5–T for every treatment. Forty-eight plants were divided into 3 groups of 16 each, each group receiving one of the three previously mentioned carriers. To the carrier for each group was added 2 percent of either an anionic or nonionic emulsifier, each being used on 8 of the 16 plants in the group. Centrally located leaves (numbers 4–7, from the base) were treated rather than basal leaves.

As in the last experiment, contact injury to the treated leaves after 2–3 days was less with the nontoxic oil-water emulsion and water only carriers than with the diesel oil-water emulsion. By the 18th day after application of the herbicide, the above differences disappeared, all treated leaves showing severe bleaching and drying. Considerable callus formation was present at this time on stems of plants treated with the nontoxic oil-water and the water only carrier, particularly in the presence of the anionic emulsifier.

One to two weeks after treatment, chlorosis began to appear in the youngest leaves, slowly advancing basipetally. In many cases bleaching or chlorosis became visible on basal leaves about 5 weeks after treatment. Considering the three different carriers, a smaller portion of plants showed foliar injury distal to the treated leaves in the case of the diesel oil-water emulsion than in the case of either of the other two carriers (Table 2). In this and the other measurements shown, no significant differences between emulsifiers appeared. Consequently, the two emulsifiers were grouped, giving 16 plants for each type of carrier.

As indicated in Table 2, petioles generally remained green when the leaves had been treated with 2,4,5–T carried in diesel oil-water emulsion, even after the leaflets and rachises had died and abscissed. However, petioles usually died along with the entire leaf after application of 2,4,5–T in a non-toxic oil-water or water only carrier. Since the petioles were not treated, the fact that they tended to remain green with the diesel oil carrier suggests a contact toxicity sufficient to inhibit herbicidal movement out of the leaflets or rachises.

Sprouting was evident in nearly all plants, but the distribution

*Table 2.* Effect of carrier on translocation, petiole mortality, and sprouting at indicated time intervals after treatment of central leaves. Herbicide was 2500 ppm. of the triethylamine salt of 2,4,5–T.

| Carrier | Number of plants showing formative effect to the untreated foliage after time indicated | | Mortality rate of petioles of treated leaves (all plants) after 25 days | | Distribution and total number of sprouts on stems after 3 months | | | |
|---|---|---|---|---|---|---|---|---|
| | Apical bud and young leaves, 25 days | Basal leaves, 5 weeks | Number petioles living | Number petioles dead | Above treated leaves | Adjacent to treated leaves | Below treated leaves | Total |
| Nontoxic oil-water emulsion 1:4..... | 15 | 16 | 16 | 48 | 17 | 2 | 16 | 35 |
| Diesel oil-water emulsion 1:4.... | 4 | 7 | 49 | 15 | 4 | 15 | 10 | 29 |
| Water..... | 14 | 12 | 7 | 57 | 11 | 6 | 10 | 27 |
| Controls (not treated)..... | 0 | 0 | 64 | 0 | 0 | 0 | 0 | 0 |

and intensity was variable, depending upon the carrier (Table 2). The most significant factor would appear to be the reduced number of stem sprouts at nodes of leaves treated with the nontoxic oil-water emulsion carrier, in spite of the fact that the overall number of sprouts resulting from this treatment was maximum. Untreated controls did not sprout.

Apical growth of the plants was rapidly and significantly repressed with all treatments, when compared with the controls. Measurements of the plants during three periods subsequent to herbicidal application (Table 3), and an analysis of variance indicate no significant difference among any of the various treatments, with the exception of carrier during the third period. Here, growth of all plants treated with the diesel oil-water emulsion was significantly (1 percent level) greater than plants receiving the other treatments, but was still well under the controls.

*Table 3.* Effect of carrier and emulsifier on apical growth of mesquite seedlings at various intervals following application of 2,4,5–T (2500 ppm.) to four centrally located leaves.

| Growth interval | Nontoxic oil-water emulsion, 1:4 | | Diesel oil-water emulsion, 1:4 | | Water | | Untreated controls |
|---|---|---|---|---|---|---|---|
| | Nonionic emulsifier[1] | Anionic emulsifier[1] | Nonionic emulsifier | Anionic emulsifier | Nonionic emulsifier | Anionic emulsifier | |
| | cm. | cm. | cm. | cm. | cm. | cm. | cm. |
| 2/4–2/19 (15 days) | 1.4±0.1[2] | 0.8±0.1 | 1.5±0.7 | 1.6±0.4 | 1.0±0.1 | 0.7±0.2 | 8.6±1.0 |
| 2/19–3/5 (14 days) | 0.0±0.1 | −0.1±0.0 | 0.5±0.4 | 0.4±0.3 | 0.0±0.0 | 0.3±0.3 | 7.9±1.4 |
| 3/5–4/2 (28 days) | −0.2±0.0 | −0.2±0.1 | 6.7±2.0 | 5.2±2.3 | 0.7±0.8 | 2.2±2.4 | 17.5±4.6 |

L.S.D. for 3rd growth interval (all treatments, excluding controls): 4.2 cm. (1 percent level) and 3.2 cm. (5 percent level). No treatment resulted in significantly different growth during the first two intervals.

[1]All emulsifiers used at 2 percent (v/v).
[2]Figures are mean of 8 plants and standard error.

Of the 48 treated plants, only 2 eventually died. One was treated with nontoxic oil-anionic emulsifier combination and the other with water-nonionic emulsifier.

A more extensive survey of various classes of emulsifying agents was next made, utilizing in every case the 1:4 emulsion of nontoxic oil in water and 2,500 ppm. triethylamine salt of 2,4,5–T which had previously given such effective results. Emulsifying agents were added to give a 2 percent concentration, except as noted in Table 4.

One hundred and twenty plants were selected for uniformity, and the herbicide was applied in the morning to four centrally located leaves of each plant. The entire experiment was replicated so that a total of 8 plants were used for each treatment. All responses described refer to the majority of plants (at least 6 out of 8), unless otherwise specified in the column heading (Table 4). Where certain responses were variable in intensity, the category is so marked. Stability of the different emulsions ranged from 1 to 200 minutes, after 10 ml. were vigorously shaken in a test tube. Although no specific correlation existed between stability and effectiveness, the 8 herbicidal emulsions giving optimum effect had a maximum stability period of 8 minutes or less.

During the 3 months following herbicidal application, terminal growth in essentially all treatments was completely repressed or nearly so. In 27 of the 29 treatments, the extreme range of mean growth was from $-0.5 \pm 0.5$ cm. to $2.0 \pm 1.7$ cm., whereas the controls grew $18.7 \pm 1.3$ cm. during the same period. As to length of stem killed back from the apical bud (Table 4), the least significant difference was 11.2 cm. at the 5 percent level of significance and 14.7 at 1 percent. In this respect, there were several emulsifiers in the anionic and nonionic classes which resulted in highly significantly greater dieback than certain others.

It is the opinion of many investigators that rapid contact kill of the treated leaves is not desirable inasmuch as it destroys the conductive channels before sufficient translocation of the herbicide has taken place. The inverse correlation between condition of treated leaves and epinasty of the apical portion of the stem (with the exception of emulsifiers numbered 16–19) would tend to bear out this theory. Apical epinasty and degree of injury to the young non-treated leaves are good indicators of the translocation of herbicides (or their toxic metabolic products) to such portions of the plant. Basal, non-treated foliage generally showed a proportional but less intense degree of injury than apical foliage, and it appeared only at a later date. These facts suggest that apical herbicidal translocation is of a greater magnitude than basal movement.

We may assume that the ideal herbicide results in the following responses: (1) repression of growth and sprouting, (2) maximum epinasty and later dieback of the stem, (3) maximum injury to distal non-treated foliage, and (4) a high percentage of ultimate plant kill. Judging by the above criteria, herbicides formulated with emulsifiers numbered 3, 4, 6, 7, 21, 22 and 28 were most effective. These emulsifiers are scattered through 3 of the 5 listed ionic classifications

*Table 4.* The effect of different emulsifying agents upon certain responses of mesquite seedlings. Triethylamine salt of 2,4,5–T,

*Table 4.* The effect of different emulsifying agents upon certain responses of mesquite seedlings. Triethylamine salt of 2,4,5–T, 2,500 ppm. carried in a nontoxic oil-water emulsion was applied to the centrally located leaves.

| Emulsifier classification (chemical nature of individual agents listed in footnote)[3] | Plant response 5 days after treatment | | Condition of young leaves 1 month after treatment | Length of stem (mean and standard error) killed back from apex after 2 months (cm.)[2] | Plant response 3 mos. after treatment | |
|---|---|---|---|---|---|---|
| | Degree of epinasty in apical portion of stem[1] | Degree of bleaching of treated leaves | | | Intensity of callus formation on stem[1] | Number of living plants sprouting |
| *Anionic* | | | | | | |
| 1 | Severe, 3 | Moderate | Variable | 9.4 ± 3.0 | Moderate, 6 | 5 of 8 |
| 2 | Moderate, 1 | Severe | V. severely chlorotic | 11.9 ± 5.0 | Slight, 4 | 3 of 7 |
| 3 | Severe, 1 | Slight | All dead | 22.0 ± 5.9 | Moderate, 3 | 1 of 5 |
| 4 | Severe, 6 | Slight | All dead | 25.8 ± 5.4 | Moderate, 4 | 0 of 6 |
| 5 | Severe, 2 | Slight | Variable | 11.3 ± 3.4 | Severe, 4 | 3 of 7 |
| 6 | Moderate, 4 | Slight | Very severely chlorotic | 16.0 ± 1.6 | Moderate, 4 | 2 of 7 |
| 7 | Very severe, 1 | Slight | All dead | 16.0 ± 3.3 | Severe, 2 | 1 of 5 |
| 8 | None | Severe | Slightly chlorotic | 0.2 ± 0.5 | Moderate, 5 | 5 of 8 |
| 9 | Slight, 2 | Severe | Variable | 2.8 ± 3.7 | Moderate, 2 | 5 of 8 |
| 10 | Moderate, 1 | Moderate | Variable | 5.0 ± 3.4 | Severe, 7 | 4 of 8 |
| *Cationic* | | | | | | |
| 11 | None | Severe | Green | 2.4 ± 2.7 | None | 4 of 8 |
| 12 | Moderate, 3 | Very severe | Severely chlorotic | 9.6 ± 7.2 | Slight, 2 | 7 of 7 |
| 13 | None | Very severe | Slightly chlorotic | 8.6 ± 6.3 | Slight, 4 | 3 of 7 |
| *Nonionic* | | | | | | |
| 14 | Moderate, 4 | Moderate | Variable | 15.0 ± 2.2 | Moderate, 3 | 4 of 7 |
| 15 | Slight, 2 | Very severe | Green | 2.6 ± 2.2 | None | 6 of 8 |
| 16 | Severe, 1 | Very severe | Moderately chlorotic | 0.5 ± 5.4 | Severe, 2 | 6 of 8 |
| 17 | Slight, 2 | Slight | Severely chlorotic | 15.2 ± 4.5 | Moderate, 4 | 4 of 8 |
| 18 | Severe, 3 | Severe | Slightly chlorotic | 16.8 ± 1.1 | Slight, 2 | 4 of 8 |
| 19 | Severe, 3 | Severe | Very slightly chlorotic | 5.9 ± 1.4 | Severe, 2 | 7 of 8 |
| 20 | None | Severe | Variable | 0.2 ± 1.0 | Moderate, 4 | 5 of 7 |
| 21 | Severe, 2 | Moderate | Very severely chlorotic | 18.7 ± 2.0 | Moderate, 2 | 1 of 6 |
| 22 | Severe, 3 | Very slight | Very severely chlorotic | 18.1 ± 4.0 | Variable | 4 of 8 |
| 23 | Moderate, 1 | Moderate | Moderately chlorotic | 4.9 ± 2.4 | Slight, 4 | 6 of 8 |
| 24 | None | Severe | Variable | 3.8 ± 1.8 | Slight, 2 | 2 of 8 |
| 25 | None | Severe | Moderately chlorotic | 1.9 ± 4.2 | Moderate, 3 | 5 of 7 |
| *Anionic-nonionic* | | | | | | |
| 26 | Severe, 1 | Moderate | Variable | 10.2 ± 6.0 | Very slight, 2 | 6 of 6 |
| 27 | Moderate, 2 | Moderate | Moderately chlorotic | 3.4 ± 4.3 | Moderate, 4 | 3 of 8 |
| 28 | Moderate, 2 | Slight | V. severely chlorotic | 14.7 ± 5.1 | Slight, 6 | 2 of 6 |
| *Cationic-nonionic* | | | | | | |
| 29 | None | Severe | Slight bleaching and chlorosis | 10.2 ± 5.4 | Moderate, 4 | 4 of 8 |
| Controls—No Herbicide | None | ——— | Green | ——— | None | 0 of 8 |

[1]Figures denote the number of plants out of 8 in which epinasty or callus occurred.

[2]L.S.D. = 14.7 cm. (1 percent level) and 11.2 cm. (5 percent level).

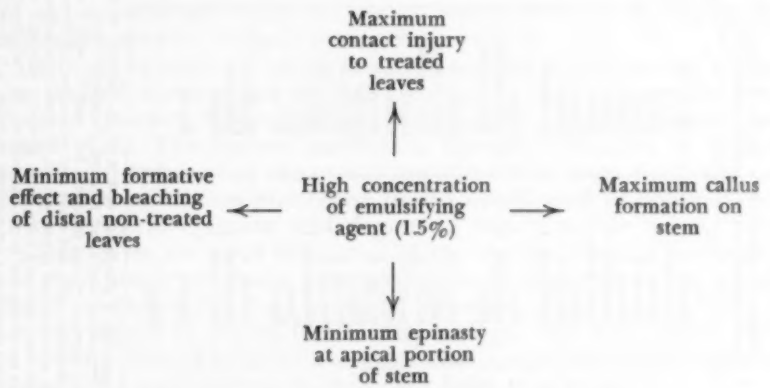
[3]Emulsifiers used at 2 percent except numbers 3–7, and 21, which were used at 0.3 percent. Chemical nature of emulsifiers are as follows: (1) Amine neutralized alkyl aryl sulfonate, (2) Blend of alkyl and alkyl aryl sulfonates, (3) Petroleum sulfonates, free and combined fatty acids and mineral oil, (4) Petroleum sulfonates, free and combined fatty acids, silicones and petroleum oil, (5)Petroleum sulfonates, free and combined fatty acids, polymers of normal iso-olefins and light petroleum oil, (6) Petroleum sulfonates, free and combined fatty acids, synthetic polymers of normal iso-olefins and light petroleum oil, (7) Petroleum sulfonates, free and combined fatty acids, polymers of normal iso-olefins and 60 vis. mineral oil, (8) Polyglycol ester—sulfonate blend, (9) Polyglycol ether—sulfonate blend, (10) polyglycol ether—sulfonate blend, (11) Di-coco dimethyl quaternary ammonium chloride, (12) Di-substituted condensate of 5 mols of ethylene oxide with soya amine, (13) Di-substituted condensate of 5 mols of ethylene oxide with tallow amine, (14) Mixed ester of fatty acids and rosin acids, (15) Triton X-100 substitute, (16) Condensate of 5 mols of ethylene oxide with mixed fatty and rosin acids, (17) Condensate of 10 mols of ethylene oxide with mixed fatty and rosin acids, (18) Essentially similar to No. 17 but more resistant to acidic or basic hydrolysis, (19) Condensate of 3 mols of ethylene oxide with coco fatty amide, (20) Condensate of 5 mols of ethylene oxide with hydrogenated tallow amide, (21) Alkylarylpolyoxyethylene glycols, free fatty acids and petroleum oil, (22) Condensate of about 150 mols of ethylene oxide with a polyoxypropylene base, total m. w. aprox. 8000, (23) Condensate of 20 mols of ethylene oxide with a polyoxypropylene base, (24) Condensate of about 9 mols ethylene oxide with a polyoxypropylene base (25) Condensate of 30 mols ethylene oxide with a polyoxypropylene base, (26) Blend of hydrocarbon sulfonates with fatty polyglycols, (27) A blend of polyoxyethylene esters of mono and dicarboxylic acids plus oil-soluble sulfonates, (28) A blend of polyglycol esters and sodium petroleum sulfonates, (29) Mono rosin acid ester of polyoxyethylene glycol.

and do not appear to have a common chemical nature. Further studies involving a specific emulsifier blend of varying concentration and hydrophile-lipophile balance would be desirable.

The oil emulsion used in all treatments is completely nontoxic (by visual determination) to the plant in itself. Likewise, several of the emulsifying agents applied at the concentration used and in the same emulsion (but without herbicide) indicated nearly complete lack of phytotoxicity. Although not every emulsifier was so tested, it is felt that differences in contact injury to treated leaves were primarily a result of variable absorption of the herbicide as brought about by the emulsifying agent, rather than toxic effect of the emulsifier itself.

In order to study the effect of emulsifier concentration upon absorption and translocation, another experiment was set up utilizing two emulsifying agents (one anionic and the other an anionic-nonionic blend), each at 0.0, 0.1, 0.4, and 1.5 percent. The triethylamine salt of 2,4,5–T was used at 5,000 ppm. in all treatments and was applied either in an emulsion of 1 part nontoxic oil to 4 parts of water or in water only. Eight plants were used in each of the 14 possible combinations of emulsifier (type and concentration) and carrier, as shown in Table 5 along with experimental results. Leaves 5–8, only, were treated during the morning hours. Control plants as usual, exhibited no discoloration or formative effect during the period. The anionic emulsifier was tested for phytotoxicity at 1.5 percent, and failed to cause contact injury *per se*.

Certain correlations between emulsifying agent, concentration, and responses occurred in the majority of cases. Schematically, these are as follows:


Maximum contact injury to treated leaves
Minimum formative effect and bleaching of distal non-treated leaves
High concentration of emulsifying agent (1.5%)
Maximum callus formation on stem
Minimum epinasty at apical portion of stem

Contact injury to the treated leaves increased with increasing concentration of emulsifying agent, as shown in Figure 3. Although the median concentration of emulsifier produced minimum stem callus, it resulted in maximum injury to the young and old non-treated leaves. To further check this inverse relationship between callus formation and injury to distal foliage, a count was taken of all 112

*Table 5.* Effect of carrier and emulsifying agent concentration upon absorption and translocation of 2,4,5–T, as exemplified by formative effects to distal foliage. The triethylamine salt of 2,4,5–T at 5,000 ppm. was applied to centrally located leaves in every case.

| Carrier, emulsifying agent, and concentration | Degree and description of bleaching of treated leaves 2 days after application of herbicide | Degree of epinasty in apical portion of stem 6 days after treatment[1] | Plant response 16 days after treatment | | Intensity of callus formation on stem[3] |
|---|---|---|---|---|---|
| | | | Condition of young leaves[2] | Degree of chlorosis of old leaves[2] | |
| **Nontoxic oil-water emulsion (1:4) as carrier** | | | | | |
| No emulsifying agent | No visible injury | None | Very slight chlorosis, 14 | No visible injury, 32 | None |
| Anionic emulsifier[4]....0.1% | Very slight, spotted | Severe, 6 | Severe chlorosis and shriveling, 2 | Slight, 14 | Moderate, 5 |
| Anionic emulsifier[4]....0.4% | Slight, spotted | Moderate, 3 | Very severe shriveling, 0 | Very slight, 22 | Slight, 2 |
| Anionic emulsifier[4]....1.5% | Slight, spotted | Slight, 2 | Severe chlorosis and shriveling, 6 | Moderate, 9 | Severe, 5 |
| Anionic-nonionic emulsifier[5]....0.1% | Very slight, spotted | Moderate, 3 | Severe chlorosis or shriveling, 1 | Moderate, 29 | Moderate, 3 |
| Anionic-nonionic emulsifier[5]....0.4% | Slight, spotted | Moderate, 3 | Very severe shriveling, 2 | Severe, 16 | Moderate, 3 |
| Anionic-nonionic emulsifier[5]....1.5% | Moderate, spotted | Moderate, 2 | Moderate chlorosis and shriveling, 4 | Slight, 19 | Moderate, 4 |
| **Water, only, as carrier** | | | | | |
| No emulsifying agent | Very slight, spotted | Slight, 2 | Moderate chlorosis, 3 | Slight, 18 | Slight, 2 |
| Anionic emulsifier[4]....0.1% | Slight, spotted | Moderate, 4 | Slight chlorosis and shriveling, 7 | Very slight, 30 | Moderate, 4 |
| Anionic emulsifier[4]....0.4% | Moderate, spotted | Slight, 1 | Moderate chlorosis or shriveling, 4 | Slight, 19 | Slight, 3 |
| Anionic emulsifier[4]....1.5% | Severe, general | Slight, 3 | Very slight chlorosis, 29 | No visible injury, 30 | Severe, 5 |
| Anionic-nonionic emulsifier[5]....0.1% | Slight, general | Moderate, 6 | Slight chlorosis and shriveling, 9 | Very slight, 25 | Slight, 4 |
| Anionic-nonionic emulsifier[5]....0.4% | Moderate, general | Moderate, 3 | Moderate chlorosis or shriveling, 2 | Moderate, 16 | Slight, 4 |
| Anionic-nonionic emulsifier[5]....1.5% | Severe, general | Slight, 3 | Slight chlorosis or shriveling, 10 | No visible injury, 27 | Slight, 4 |

[1]Figures denote number of plants out of 8 on which epinasty occurred.
[2]Figures denote number of young and old leaves, respectively (per 8 plants) remaining green 5 weeks after treatment.
[3]Figures denote number of plants per 8 on which callus was present 5 weeks after treatment.
[4]Petroleum sulfonates, free and combined fatty acids, polymers of normal iso-olefins and 60 viscosity mineral oil.
[5]Polyglycol ester and sodium petroleum sulfonates in an inert base.

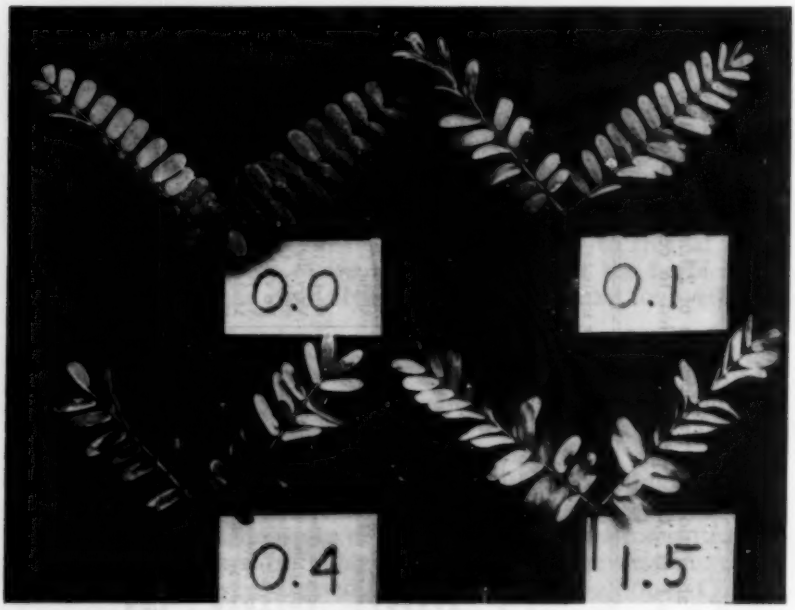



FIG. 3. Relation of contact leaf injury, five days after treatment, to percentage concentration of emulsifier, as shown. Herbicide was 5,000 ppm. of the triethylamine salt of 2,4,5–T in every case, with water as the carrier.

plants, regardless of treatment. Of those developing callus, 48 percent showed a certain amount of injury to the apical portion 3 weeks after treatment, whereas of those without visible callus, 83 percent exhibited marked injury (Figure 4). The presence of callus thus appears to be associated with an effective blocking of herbicidal movement beyond the calloused region.

Callus generally formed on the stem adjacent to nodes of the treated leaves, with the exception of formulations involving the highest concentration of emulsifying agent. In the latter case, callus generally extended well into the apical portion of the stem as well.

One point of interest was that 2,4,5–T carried in the nontoxic oil-water emulsion with 0.4 percent emulsifier (either type) resulted in rapid drying and shriveling of the apical leaves, before chlorosis could set in. The higher and lower concentrations of emulsifier first resulted in chlorosis.

Of the 112 plants, only 16 were dead 2 months after treatment. These were randomly scattered throughout all classes. Basal sprouts of plants still living averaged one per plant and also appeared at random throughout all treatments.

The results of this experiment indicate the comparative importance of proper emulsifier concentration. It is very likely that the range of optimum concentration for a specific plant species is quite

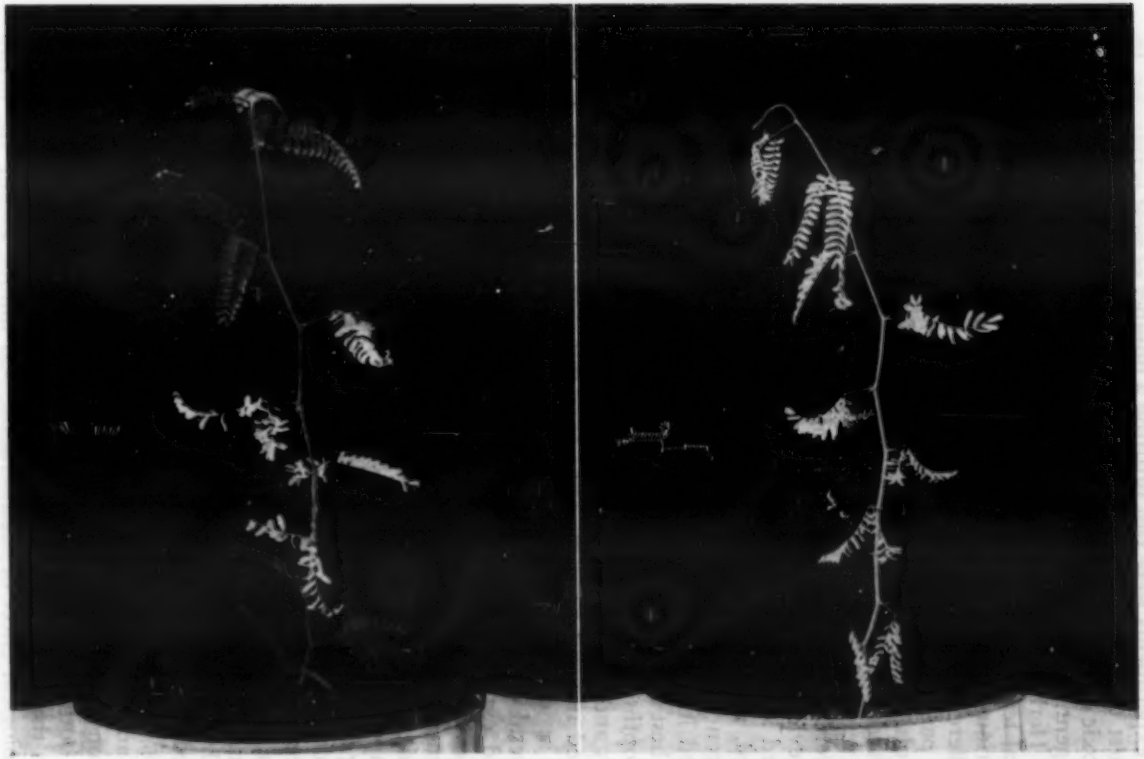


FIG. 4. Plants treated on four centrally located leaves with 5,000 ppm. of the triethylamine salt of 2,4,5-T in a water carrier. Herbicide applied to left plant was fortified with 1.5 percent emulsifier, while that applied to the right plant received 0.1 percent emulsifier. Note lack of callus on the latter plant, but pronounced injury to the apical and basal leaves.

narrow, and that improper concentrations are commonly utilized in field applications.

*The Effect of Several New Herbicides upon Mesquite Seedlings.* Two experiments were run utilizing a number of new herbicides. In the first trial, all herbicides were employed at 2,500 ppm. in a 1:4 nontoxic oil-water emulsion, with the addition of 2 percent emulsifying agent. Eight plants per treatment were used in replicates of 4 each. Herbicidal application was made during the morning to the central leaves (numbers 4–7, from base). Responses which occurred at various intervals following treatment are shown in Table 6.

Differences between the means of the terminal growth are for the most part small and nonsignificant, except when compared to the controls. With the exception of the butoxyethanol ester of 3,4–D, which gave highly variable degrees of growth repression, all herbicides resulted in nearly complete repression.

Severe contact injury to the treated leaves is probably undesirable, as previously mentioned, since conductive tissue may thus be destroyed and inhibit further movement of the herbicide. That this, indeed, must occur is borne out by the inverse correlation found between the degrees of injury to treated leaves and to distal nontreated leaves. It would appear that free acid granules of 2,4,5–T gave best results under the conditions of this experiment, assuming that optimum herbicidal activity is achieved by minimum contact injury and minimum sprouting, accompanied by maximum epinasty, marked injury to non-treated leaves and growth repression or dieback, as well as maximum kill.

Several other herbicides, some relatively new, were included in a second experiment of similar design, with the exception that the herbicidal concentration was raised to 5,000 ppm. and the emulsifier concentration lowered to 0.2 percent (1 percent in the case of 2,5–D, in order to maintain a moderately stable emulsion). The herbicide was applied to three centrally located leaves per plant, 10 plants being used for each treatment. Responses which followed are recorded in Table 7.

Sprouting generally occurred from one or more of the basal four nodes, with the exception of aminotriazole-treated plants, where sprouting was randomly distributed at all nodes (Figure 5). Two months after treatment, all plants were cut off 5 mm. above the cotyledonary node. Sprouting from this node occurred in nearly all plants, including the controls. However, on plants previously treated with aminotriazole sprouting occurred earlier and more profusely than on those of any of the other treatments. All 10 plants sprouted from both sides of the cotyledonary node, whereas with other treatments the majority sprouted from only one side of the node. The physiological effect of aminotriazole was unique among the herbicides tested, and the need for further investigation at different concentrations and in combination with other materials is certainly indicated.

*Table 6.* Certain responses of mesquite seedlings resulting from application of 2,500 ppm. of the herbicide indicated to four centrally located leaves. Carrier 1:4 emulsion of nontoxic oil and water with 2 percent emulsifying agent.

| Herbicide | Plant response 5 days after treatment | | Plant response 1 month after treatment | | Length of stem killed back from apex, 2 months after treatment (cm.) | Plant response 3 months after treatment | |
|---|---|---|---|---|---|---|---|
| | Severity of apical epinasty and its occurrence in number of plants out of 8 | Degree of bleaching of central treated leaves | Condition of young non-treated leaves | Condition of old non-treated leaves | | Intensity of callus formation on stem and its occurrence in number of plants per 8 | Number of plants sprouting of those living |
| 2,4-D, free acid colloidally ground | Mod. 3 | Slight | Very slightly chlorotic | Very slightly chlorotic | 12.6 ± 4.6[1] | Slight, 5 | 3 of 8 |
| 3,4-D butoxy-ethanol ester | Mod. 1 | Very severe | Green | Green | 2.5 ± 1.6 | None | 7 of 8 |
| 2,4,5-T emulsive free acid | None | Very severe | Very slightly chlorotic | Green | 0.8 ± 0.5 | None | 3 of 8 |
| 2,4,5-T granules of free acid | Mod. 2 | Very slight | Mod. to severe chlorosis | Slightly chlorotic | 17.6 ± 3.0 | Mod. 3 | 0 of 7 |
| 2,4,5-T butoxy-ethanol ester[2] | Mod. 2 | Very severe | Slightly chlorotic | Green | 0.0 ± 2.4 | None | 4 of 8 |
| 2,4,5-T butoxy-ethanol ester[2] | None | Very severe | Very slightly chlorotic | Green | 3.0 ± 2.1 | None | 2 of 8 |
| Control (no herbicide) | None | ——— | Green | Green | ——— | None | 0 of 8 |

[1]Mean and standard error.
[2]These formulations were prepared with different emulsifying agents.

*Table 7.* Certain responses of mesquite seedlings resulting from application of 5,000 ppm. of the herbicide indicated to three centrally located leaves. Carrier 1:4 emulsion of nontoxic oil and water with 0.2 percent emulsifying agent.

| Herbicide | Plant response 5 days after treatment | | Plant response 18 days after treatment | | | Length of stem killed back from apex 5 weeks after treatment[1] (cm) | Total number sprouts per number plants living 2 months after treatment |
|---|---|---|---|---|---|---|---|
| | Severity of apical epinasty and its occurrence in number of plants per 10 | Nature and severity of injury to treated leaves | Nature and severity of injury to young leaves and number of young leaves per 10 plants showing no chlorosis or formative effect | Nature and severity of injury to basal leaves and number of basal leaves per 10 plants showing no chlorosis or formative effect | Intensity of callus formation on stem and its occurence in number of plants per 10 | | |
| 3-Amino 1,2,4-triazole | None | No visible injury | None, 28 | None, 25 | None | 0 | 54/10 |
| 2,5-D, butoxy-ethoxy propanol ester | None | Small spotted slightly bleached areas | Variable, 13 | Very slightly chlorotic, 22 | Severe, 2 | 5.2 ± 1.5 | 19/9 |
| 2,4,5-T, capryl ester | Slight, 2 | Severely bleached | Moderately chlorotic and shriveled, 12 | None, 26 | Very slight, 2 | 5.3 ± 1.0 | 17/10 |
| 2,4,5-T, triethylamine salt | Moderate, 7 | Small spotted slightly bleached areas | Severely chlorotic or bleached. Very severely shriveled, 2 | Moderately chlorotic or bleached. Slightly shriveled, 8 | Slight, 3 | 11.3 ± 1.0 | 25/8 |
| 2,4,5-Trichlorophenoxypropionic acid, propylene and polypropyleneglycolbutylether esters | Moderate, 3 | Severely bleached | Moderately chlorotic or bleached. Severely shriveled, 7 | Very slightly chlorotic, 18 | None | 6.9 ± 1.4 | 14/10 |
| 2,2-Dichloropropionic acid, sodium salt | None | No visible injury | Slightly chlorotic or bleached. Very severely shriveled, 13 | Very slightly chlorotic, 19 | Slight, 1 | 3.6 ± 1.1 | 28/10 |
| Controls | None | None | None | None | None | 0 | 0/10 |

[1]Mean and standard error shown. Least significant difference 3.3 cm. (5 percent level) and 4.4 cm. (1 percent level).

In evaluating the data of Table 7 in a manner described for the previous experiment, it is apparent that optimum results are achieved with the triethylamine salt of 2,4,5–T, while the mixed ester of 2,4,5–trichlorophenoxypropionic acid follows a close second.

It should be kept in mind that each of these two experiments represents a single carrier and a single concentration of herbicide and emulsifying agent. With variations in these factors, results would undoubtedly be very different. For example, herbicides demonstrating a high contact phytotoxicity may give enhanced over-all activity, as measured by this type of test, if used at lower concentrations.

FIG. 5. Excessive sprouting created by application of 5,000 ppm. of 3–amino–1,2,4–triazole to three central leaves of the original single-stemmed plant.

## DISCUSSION

In view of the fact that field tests have generally indicated ester formulations of 2,4,5–T to give better results than the amine, sodium salt, and free acid formulations, the finding that the latter type exhibit superior translocation in greenhouse-grown seedlings is of interest. The probability exists that plants which have grown under the xeric conditions of the southwest have leaves with heavier cuticles than young seedlings. Thus, as suggested by Crafts (4, 5), it is likely that more nonpolar formulations (e.g., the oil soluble esters) would be more compatible with the waxy cuticle of the field plants and more readily absorbed. Because of the relatively thin cuticle found in the greenhouse grown seedlings, it would appear that they could be utilized as a tool for translocation studies *per se*, absorptive effects thus being minimized.

The finding that a nontoxic oil-water emulsion carrier results in superior absorption and/or translocation of herbicide than diesel oil emulsion is in line with thoughts held by numerous investigators. The contact toxicity of diesel oil is such that injury to the vascular system of the leaves may occur sufficiently rapidly to inhibit further absorption and translocation of the residual herbicide. Indeed, as described in the text, the leaf may absciss, allowing its petiole to remain as a green stump, the latter having received an insufficient supply of herbicide to show damage. With a nontoxic carrier, such abnormal abscission seldom occurs.

The relationship of marked contact injury and callus formation along with minimum apical epinasty and minimum injury to distal non-treated leaves—all associated with a relatively high (1.5 percent) concentration of emulsifying agent—is of interest. That translocation is inhibited by partial destruction of the phloem (as evidenced by callus formation), is suggested by the data presented. However, the inverse correlation between callus formation and distal foliage injury does not appear to hold in every case when varying intensities of callus are brought about by type of herbicidal formulation or emulsifier rather than by *concentration* of the emulsifying agent. Destruction of phloem in this manner has been described in detail by Eames (6) in the case of the bean plant.

It is conceivable that phloem at the central region of the stem could be partially or completely disrupted, while at the same time adjacent xylem would still remain functional, the vessels allowing transport of water and minerals to the apical portion of the plant. In this manner, photosynthates produced in the leaves just above the treated leaves could be translocated and utilized by the rapidly growing apical bud and juvenile leaves. Likewise, carbohydrates elaborated in the foliage basal to the treated leaves should be sufficient to maintain active metabolism in the roots. Indeed, plants may commonly be observed to remain in an apparent healthy state, under the above condition, for many months at a time. It is possible, of course, that the phloem does not actually block organic translocation because of its inactivation or injury. The active herbicide may fail to reach distal portions of the plant simply because of its inactivation or metabolism within localized regions of the stem, callus formation being more or less incidental.

The relationship of the effects resulting from changes in emulsifier concentration in conjunction with variable herbicidal concentrations is indeed complex, and needs further study. In the great majority of research reported on weed investigations, herbicidal concentration and carrier volume play the dominant role, with emulsifier concentration generally receiving little or no attention. From all indications, however, it would appear that the type and optimum range of concentration of emulsifier are very critical for specific herbicidal formulations and carriers used for certain specific plants.

That herbicidal absorption is enhanced by emulsifying agents has been well established (3, 8, 11, 15, 20). In rate of killing tomato plants, however, Hitchcock and Zimmerman (13) have shown that little difference exists between rather high emulsifier (Tween #20) concentrations, ranging from 1 to 5 percent, when added to a 500 ppm. solution of 2,4–D sodium salt.

In general, the work herein reported upholds the view of Crafts (5), that water soluble or polar herbicidal formulations (e.g., the sodium salt of 2,4–D) are more readily translocated within the sieve tube than aliphatic esters. The recent work of Emrick and Leonard (7), in which the amine formulation of 2,4–D was found to give outstanding, though delayed, kill of *Quercus wislizenii* after spraying

of the sprouts, would also support this point of view. An essential limiting factor to be overcome would appear to be achieving penetration of water soluble herbicides through a cuticle of lipoidal nature. A second limiting factor which must be overcome to obtain good root kills is the achievement of sufficient basal translocation before phloem in the aerial portion of the plant is completely disrupted. Careful selection of emulsifying agents as to their proper type, hydrophile-lipophile balance, and correct concentration, should be one method of partially overcoming these limiting factors.

## SUMMARY

Absorption and translocation in velvet mesquite seedlings was studied by painting various formulations of herbicides in different carriers on three or four centrally located leaves, and noting subsequent formative effects in other portions of the plant. Other criteria used as a measure of absorption and translocation were epinasty, growth repression, dieback, and sprouting.

The use of a nontoxic oil in a 1:4 oil-water emulsion as a carrier for 2,4,5–T resulted in considerably greater injury to the non-treated distal foliage than did diesel oil.

When carried in a nontoxic oil emulsion, the free acid, the triethylamine and sodium salts of 2,4,5–T all demonstrated a greater tendency to be translocated to more distant portions of the plant than did ester formulations, even though contact injury to the treated leaves was less.

Various herbicidal adjuvants, including carbohydrates and latex emulsion gave results not significantly different from the controls containing herbicide and emulsifying agent only.

Plants given a pre-dark period before treatment with an ester of 2,4,5–T responded similarly to those not given the dark period. On the other hand, of the pre-darkened plants treated with the sodium salt of 2,4,5–T, less contact injury to the leaves was noted than in the case of the non-darkened plants. This difference, however, was considerably less than the difference in marked contact injury caused by the ester as compared to the sodium salt.

Type and concentration of emulsifying agent was found to be of particular importance for maximum absorption of herbicide. Concentrations above 0.4 percent of certain emulsifiers resulted in callus formation on the stem adjacent to nodes of the treated leaves. Further herbicidal translocation beyond the callus was effectively blocked, as evidenced by lack of formative effect to the apical bud and leaves and to the basal leaves. The importance of the relationship of herbicide and emulsifying agent concentrations is discussed.

Of a number of relatively new herbicides tested, none was found to give more effective results than the water-soluble formulations of 2,4,5–T.

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# The Carbohydrate Content of Corn Plants as Affected by Isopropyl N–(3–Chlorophenyl) Carbamate[1]

J. A. Meade and A. O. Kuhn[2]

## Introduction

The herbicide, isopropyl N–(3–chlorophenyl) carbamate (CIPC), has proved to be efficient in eliminating certain weeds from several crops. The toxic mechanism of this herbicide is still somewhat doubtful. In order to further determine the reaction of plants to this herbicide the carbohydrate content of corn plants sprayed with CIPC was investigated.

## Review of Literature

Several reviews of the physiological and herbicidal activity of isopropyl N–phenyl carbamate (IPC) have appeared recently in the Annual Review of Plant Physiology (3, 5, 17).

The first evidence that carbamates injure plants was reported in an article by Lefevre (14). He showed the similarity of the action of carbamates to colchicine. Ivens and Blackman (12), using ethylphenylcarbamate, found that in treated barley and pea roots a disruption of mitosis with accumulation of arrested metaphase states occurred. Ennis (6), using IPC treatments on *Avena* and *Allium*, found mitotic aberrations similar to those noted by Lefevre on wheat. Ennis also found that application of IPC to the roots of *Avena* produced histological changes in the shoot meristem indicating transport of the substance or stimulus from the root to the apical meristem.

The antimitotic effects of the carbamates are considered to be the principle mechanism involved in killing treated plants. Ivens and Blackman (13), however, have suggested that some metabolic activity connected with mitosis may be affected.

Later, Freed (9) stated:

> "The deep green color resulting from treatment with isopropyl phenylcarbamate is directly due to an increased amount of chlorophyll in the tissues. Treated plants will contain from 19 to 28% more chlorophyll per unit leaf area or per gram of dry weight than comparable untreated plants."

Freed also found that "reserve carbohydrates of the plant are much lower than the control, although reducing sugars at any given moment will be higher".

Swanson, Shaw, and Hughes (20) have shown that the respiration of cotton roots grown in contact with CIPC is progressively depressed with an increase in concentration. They also have shown, that, when previously untreated cotton roots are brought into contact with CIPC, the $O_2$ uptake is depressed more than $CO_2$ evolution with a

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resultant high RQ indicating the possibility that anaerobic fermentation was induced.

Tomizawa and Koike (21) studied the effect of IPC on several enzymes and carbohydrate metabolism of rice seed, rice seedlings, and sweet potato. They found that IPC decreased phosphatase activity and lowered the amount of phosphate in rice seeds. In rice seedlings phosphatase activity was increased with a corresponding increase in the amount of phosphate present. The phosphatase activity of sweet potato was not affected. Amylase activity in rice seedlings was greatly increased in rice seedlings as was invertase. The increase in reducing sugars, however, was not proportional to the increased activity of amylase and invertase. Sucrose did show a decrease proportionally with the increased invertase activity. Reducing sugars in rice seed showed an initial increase and then dropped rapidly. In sweet potato the treated plants had about the same amount of reducing sugars as the untreated control.

Tomizawa and Koike also determined that catalase and peroxidase activity were decreased in both rice seedlings and sweet potato with treatment by IPC.

The effects of IPC as reported by Tomizawa anud Koike indicated that enzyme systems connected with carbohydrate metabolism are profoundly affected by IPC. Their determinations of reducing sugars and sucrose show that carbohydrate metabolism was directly affected.

Although most of the early work in weed control with carbamates was with IPC it was not long before the greater activity of the 3–chloro derivative was discovered. Ennis (7) and Witman and Newton (23) reported the more toxic effect of CIPC. Since then considerable work has been done with CIPC as an herbicide. It has been shown (22) that CIPC, used as a pre-emergence spray, is quite effective in controlling weedy grasses in cotton anad soybeans. CIPC has been reported to be effective in controlling chickweed in seedling alfalfa (1, 16, 24). In the West, CIPC is used extensively in controlling weedy grasses in seed crops of grasses and legumes (8).

## PROCEDURE

Corn was chosen as a test plant because it is very susceptible to CIPC and yet sub-lethal dosages show effects. The hybrid U. S. 13 was used in the study.

The corn was planted in greenhouse flats and grown under near optimum conditions for corn growth. Seventy-five seeds were planted per flat.

The herbicide was applied as a pre-emergence spray in acetone using the equipment described by Shaw and Swanson (18). The herbicide was sprayed at the rates of 0, 2, 4, and 8 pounds per acre of active ingredient.

The flats were watered immediately after spraying with 500 ml. of water per flat. Subsequent water was applied by sub-irrigation techniques. Moisture was maintained at optimum conditions throughout the experiment.

The plants were sampled 10 days after planting. At this time they were 16 inches tall. In each of the treated flats there were some plants which showed signs of injury and others which appeared uninjured. These were harvested and analyzed separately. The plants were cut off at or just below the soil line and killed in boiling 95 per cent ethanol.

Extraction of the carbohydrates was carried out by the soxhlet method as described by Loomis and Shull (15). Two hundred-fifty ml. of the ethanol extract was converted to a water extract. This extract was cleared with neutral lead acetate and deleaded with dipotassium phosphate. The ceric sulfate method for reducing sugar determinations as described by Hassid (10, 11) and modified by Barr (2) was used. Reducing sugars, invert sugars, and hemi-reserve polysaccharides were determined.

## EXPERIMENTAL RESULTS

### *Effect of CIPC on Reducing Substances.*

The plants which were treated with CIPC and which showed definite injury were found to have highly significant increases in reducing substances as compared to the untreated controls at all rates of application of the herbicide. This is shown in Table 1 and Figure 1. There were no significant differences within the rates of CIPC, although the 2-pound rate resulted in an increase in reducing substances over the 4- and 8-pound rates. The CIPC treated, but apparently uninjured plants, showed no significant differences in reducing substances over the untreated control. The 8-pound rate did, however, show a slight increase, as shown in Figure 2.

*Table 1.* The results of carbohydrate determinations with corn plants grown in a greenhouse and treated with isopropyl N–(3–chlorophenyl) carbamate (CIPC) as a pre-emergence spray.

| Rate in pounds per acre of CIPC | Reducing sugars mg [1] [2] | Invert sugars mg [1] [2] | Total sugars mg [1] [2] | Hemi-reserve polysaccharides mg [1] [2] |
|---|---|---|---|---|
| Uninjured plants | | | | |
| 0 | 1.893 | 1.465 | 3.358 | 2.282 |
| 2 | 2.329 | 1.559 | 3.888 | 2.550 |
| 4 | 2.174 | 1.369 | 3.543 | 2.384 |
| 8 | 2.368 | 1.912 | 4.280 | 2.673 |
| Injured plants | | | | |
| 0 | 2.224 | 1.909 | 4.134 | 2.530 |
| 2 | 4.799 | 4.129 | 8.928 | 3.535 |
| 4 | 4.164 | 3.414 | 7.578 | 3.290 |
| 8 | 4.611 | 2.738 | 7.349 | 2.952 |
| 5% L.S.D. | 1.135 | 0.923 | 1.797 | |
| 1% L.S.D. | 1.680 | 1.288 | 2.506 | |

[1]Milligrams per gram of fresh weight.
[2]Average of triplicate determinations of 3 replicates.
[3]Single determination of 1 replicate.

### *Effect of CIPC on Invert Sugars*

The treatment at all rates of CIPC, as shown in Table 1 and Figure 1, caused a highly significant increase in invert sugars, except at

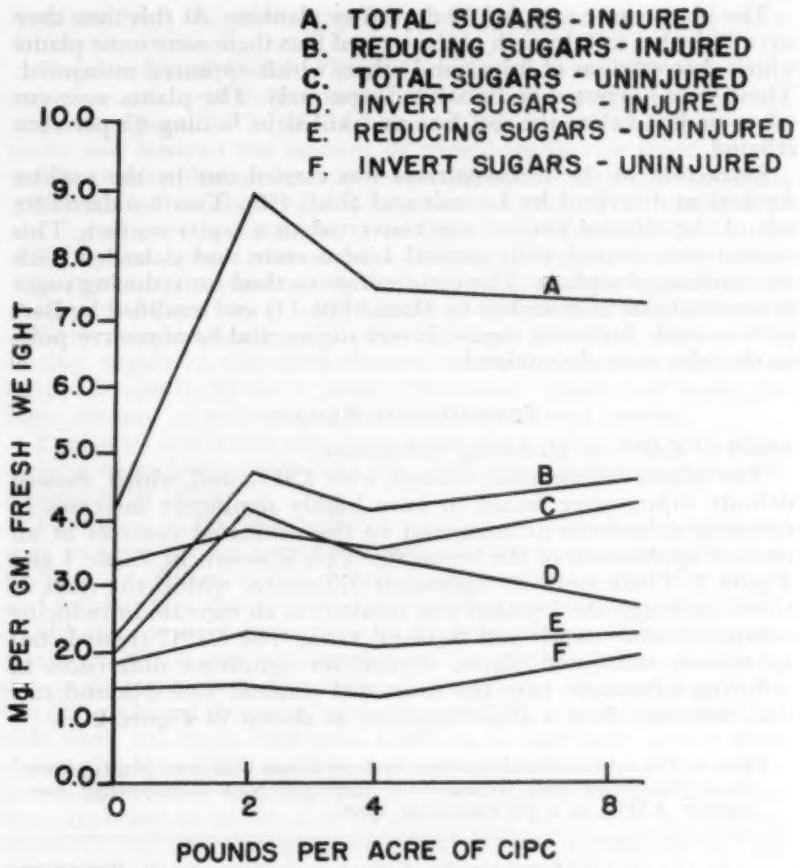


FIG. 1. The quantities of reducing sugars, invert sugars, and total sugars of uninjured and injured corn plants grown in the greenhouse and treated with isopropyl N-(3-chlorophenyl) carbamate (CIPC) as a pre-emergence spray.

the 8-pound rate which was significant. The 2-pound rate was also significantly higher in invert sugars than the 8-pound rate.

*Effect of CIPC on Total Reducing Substances*

The levels of total sugars of the treated plants, as shown in Table 1 and Figure 1, were significantly higher than check at all rates. The peak was at the 2-pound rate of CIPC per acre, but it was not significantly higher than the 8-pound rate of the herbicide.

*Effect of CIPC on Hemi-Reserve Polysaccharides*

A representative sample of the residues from the ethanolic extraction was tested for acid hydrolyzable substances. These hemi-reserve polysaccharides, as reported in Table 1 and Figure 2, showed a slight increase due to treatment with CIPC.

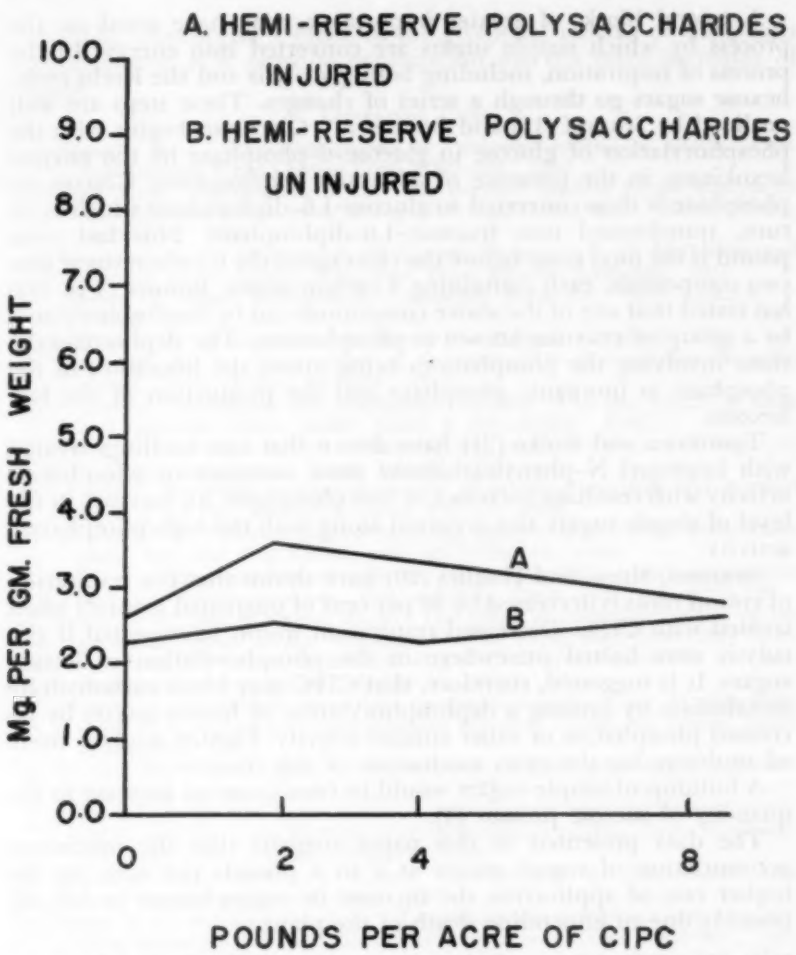


FIG. 2. The quantity of hemi-reserve polysaccharides in uninjured and injured corn plants grown in the greenhouse and treated with isopropyl N–(3–chlorophenyl) carbamate (CIPC) as a pre-emergence spray.

## DISCUSSION

The results of this experiment show that there was a distinct increase in reducing sugars and sucrose of plants treated with isopropyl N–(3–chlorophenyl) carbamate (CIPC) and a possible increase in the reserve polysaccharides. These increases would indicate that there is a block in the carbohydrate metabolism causing the increase. It is suggested that two such blocks may be responsible for these results. One, of minor magnitude, may occur in such a way that the breakdown of polysaccharides is inhibited.

A second block, of greater importance, may have acted on the process by which simple sugars are converted into energy. In the process of respiration, including both glycolysis and the Krebs cycle, hexose sugars go through a series of changes. These steps are well outlined by Stumpf (19) and Bonner (4). Glycolysis begins with the phosphorylation of glucose to glucose–6–phosphate by the enzyme hexokinase in the presence of adenosine triphosphate. Glucose–6–phosphate is then converted to glucose–1,6–diphosphate which is, in turn, transformed into fructose–1,6–diphosphate. This last compound is the final stage before the cleavage of the 6 carbon sugar into two compounds, each containing 3 carbon atoms. Bonner (4 p. 189) has stated that any of the above compounds can be dephosphorylated by a group of enzymes known as phosphatases. The dephosphorylations involving the phosphatases bring about the liberation of the phosphate as inorganic phosphate and the production of the free hexoses.

Tomizawa and Koike (21) have shown that rice seedlings treated with isopropyl N–phenylcarbamate show increases in phosphatase activity with resultant increases in free phosphate. An increase in the level of simple sugars also occurred along with the high phosphatase activity.

Swanson, Shaw, and Hughes (20) have shown that the respiration of cotton roots is decreased by 50 per cent of untreated controls when treated with CIPC. Decreased respiration would be expected if glycolysis were halted somewhere in the phosphorylation of hexose sugars. It is suggested, therefore, that CIPC may block carbohydrate metabolism by causing a dephosphorylation of hexose sugars by increased phosphatase or other enzyme activity. Further study is needed to determine the exact mechanism of this reaction.

A buildup of simple sugars would in turn cause an increase in the quantity of sucrose present (4).

The data presented in this paper suggests that the maximum accumulation of sugars occurs at 2 to 4 pounds per acre. At the higher rate of application the increase in sugars begins to fall off, possibly due to impending death of the plant.

## SUMMARY

The carbohydrate metabolism of corn plants grown under greenhouse conditions and treated with CIPC applied as a pre-emergence spray has been studied.

The principal results of this investigation were as follows:

1. The ceric sulfate method for determining reducing substances was found to be satisfactory for this study.

2. The level of reducing sugars, invert sugars, and total sugars of treated plants was significant over the controls.

3. A representative sample of the treatments in each experiment was treated for acid hydrolyzable polysaccharides. The plants treated with CIPC showed slightly higher levels of polysaccharides than did the untreated control.

4. The presence of a block in the glycolytic scheme of the plants was discussed as a possible explanation for the large increases of sugars.

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# The Use of $C^{14}$ Carbonyl Labeled 3(*p*–chlorophenyl)–1, 1–dimethyl Urea in a Leaching Study[1]

H. R. Sherburne, V. H. Freed, and S. C. Fang

The distribution of 3 (*p*–chlorophenyl)–1,1–dimethyl urea (CMU) in the soil horizon is an important factor to consider in using the material since it is a soil active herbicide. It has been reported that the persistence and toxicity of CMU vary considerably with soil type, temperature, and moisture conditions (1).

This study was conducted to determine the effect of soil moisture, precipitation, and soil type on the movement of radioactive CMU in the soil.

It should be recognized that there are at least three mechanisms by which a chemical is lost from the soil, namely: microbiological destruction, leaching and chemical loss including vaporization. The present study is an attempt to gain further insight on the contribution of leaching as a factor in the loss of CMU from the active soil zone.

## Materials and Methods

*Preparation of Tubes.* Soft glass tubes, 10 mm in diameter, were divided into sections and marked with a file ½, 1, 1½, 2, 2½, 3, and 4 inch intervals, and at one inch intervals to 12 inches.

The lower end of the tube was then covered with a piece of cheese cloth that was secured with a rubber band. This procedure was similar to that reported by Logan, Odell and Freed with IPC (3).

*Preparation of Paper Disks.* Round paper disks of S&S Black Ribbon filter paper were cut to the size of the inside diameter of the glass tubes. A 95 per cent ethyl alcohol solution containing 0.352 mg of $C^{14}$ carbonyl labeled 3 (*p*–chlorophenyl)–1,1–dimethyl urea, with a specific activity of 267,000 cts/min/mg, was then added to each disk with a micro syringe. This rate of application was equivalent to 40 pounds per acre. By using this method to introduce the CMU, the alcohol was prevented from carrying the material into the soil.

*Preparation of Soil.* The air dried soils, Chehalis sandy loam and Willamette clay loam, were separately mixed and sifted through a 40 mesh screen. Their moisture contents were determined and known amounts of these samples were placed in erlenmeyer flasks with enough water to make the predetermined moisture levels of 15 per cent (which is approximately what would be found in the summer time in many areas) and 35 per cent for the sandy loam soil and 15 and 40 per cent for the clay loam soil. These two higher levels of moisture were selected because they approached field capacity for the respective soils.

[1]Contribution from the Dept. of Agricultural Chemistry, Oregon State College, Corvallis, Oregon. Technical Paper No. 865, Oregon Agricultural Experiment Station. This work was supported in part by a grant from E. I. DuPont de Nemours and Company.

*Preparation and Leaching of Soil Column.* The tubes were uniformly packed with the prepared soil and a paper disk containing 0.352 mg CMU were placed on the top of the columns. To assure uniform extraction of CMU from the treated disk, another paper disk was put on top of the first one before the addition of water. Then the appropriate amounts of water, equivalent to 1, 3, or 9 inches of precipitation, were introduced dropwise at the top of the columns. After 24 hours, the tubes were divided into sections with a hot wire glass cutting machine.

*Combustion.* The soil sections were oxidized by the wet combustion method of Van Slyke-Folch (2) and their radioactivities were measured as described by Fang *et al.* (4). All the carbon was recovered as barium carbonate. The precipitate was filtered through a medium sintered glass crucible, washed 3 times with distilled water and once with 95 per cent alcohol, and dried at 105 to 110°C for more than two hours. A portion of this barium carbonate was deposited in a thin layer on a one inch copper disk and the radioactivity was determined with a Tracerlab autoscaler and a G-M thin mica window counter (1.9 mg/cm$^2$). All results are an average of two determinations.

## RESULTS

The amounts of CMU that were found at the different depths are presented in Figures 1 and 2. Figure 1 presents the values that were calculated from the total activity for the sandy soil and Figure 2 presents similar values for the clay soil. In Figure 1 the amount of radioactive CMU that was found in the ½ inch segments was plotted directly.

In an effort to explain the reason for the two peak concentrations (Figure 1b) in the sandy soil, it was suggested that this could be caused by some action of microorganisms. Therefore a sample of this prepared soil was autoclaved for 20 minutes at 20 lbs pressure at 257°F, dried in an oven, made up to 15 per cent moisture, and the amount of leaching determined. It was found that this sterilized soil produced the same results, with respect to leaching, as the non-sterilized soil. Consequently it was concluded that these two peaks were not caused by microorganisms.

In order to verify the fact that the $C^{14}$ labeled CMU acted the same, with respect to leaching, as pure non-radioactive CMU[2], one column was treated with pure nonlabeled CMU and the amount of leaching was determined chemically according to the procedure outlined by Lowen and Baker (5). No difference was detected in the amount of leaching of nonlabeled and labeled CMU.

To determine the effect of the paper disk method of introducing the CMU to the column, on the amount of leaching, one column of 15 per cent moisture sandy soil was determined by adding the CMU solution directly to the soil. This method had no effect on the leaching except that the alcohol carried the CMU down into

[2]99.9 per cent pure 3 (*p*–chlorophenyl)–1,1–dimethyl urea. Courtesy E. I. DuPont de Nemours & Co., Grasselli Chemicals Department, Wilmington, Delaware.

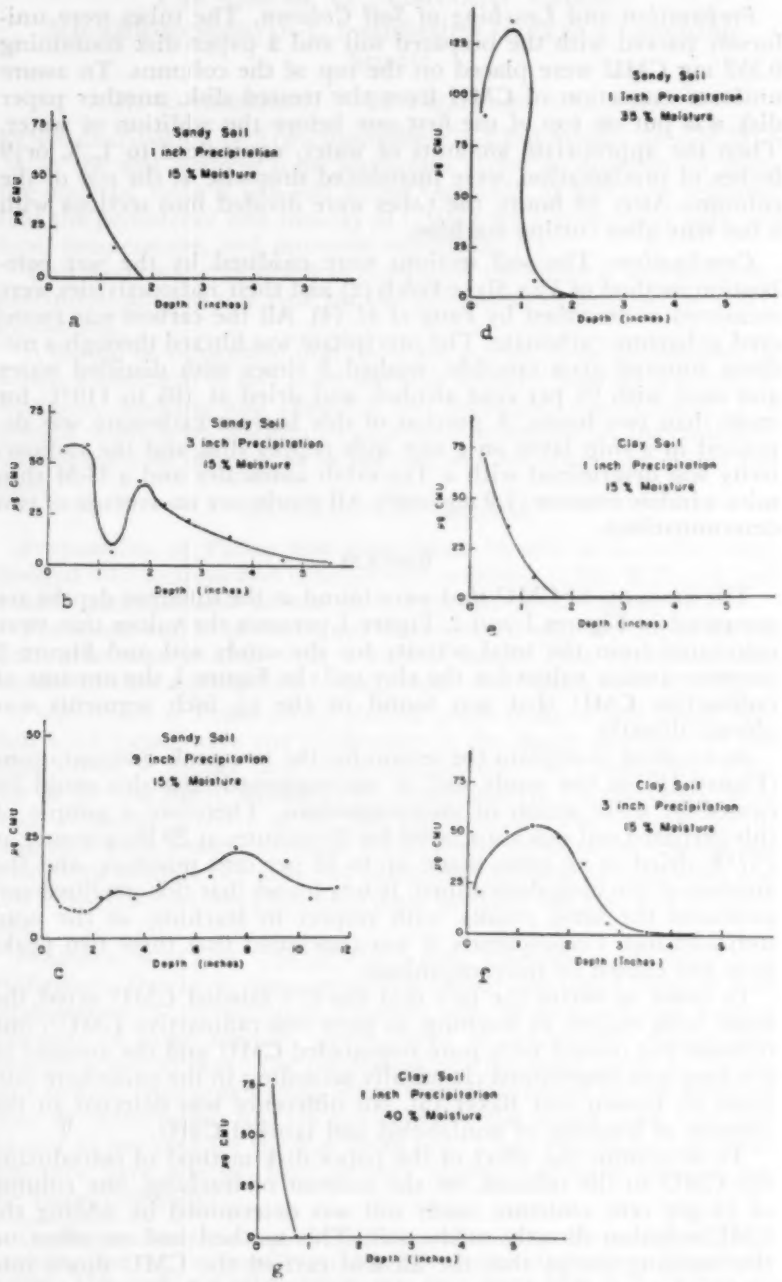


FIG. 1. Movement of CMU in two soils with two moisture levels after leaching with 1, 3 and 9 inches of water.

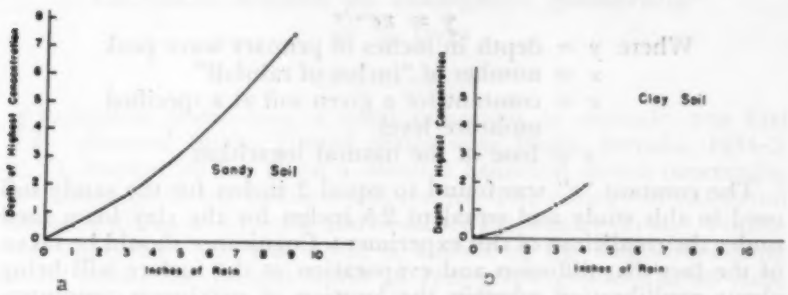


FIG. 2. Movement of highest CMU concentrations in sandy and clay soils after leaching with water.

the soil to approximately ¼ inch of depth. It was found that one inch of water moved about 35 per cent of the CMU into the soil from the disk, two inches moved 61–70 per cent and three inches 90 per cent.

## DISCUSSION

An analysis of Figures 1-a, 1-b and 1-c indicates that the maximum concentration of CMU in the soil moves down as the amount of precipitation increases. This can also be noted by comparing Figures 1-e and 1-f.

A comparison of Figure 1-a with Figure 1-d and Figure 1-e with Figure 1-g illustrates that as the amount of moisture in the soil prior to application of CMU increases, the depth of leaching decreases. This is probably attributable to a slower movement of the water through the more moist soil.

Also, a comparison of the graphs of the movement of CMU in the sandy soil with the movement in the clay loam soil shows that as the fraction of smaller particles increases, the downward movement of the herbicide decreases. This information agrees with the work that was reported by Loustalot *et al.* (1).

It has been shown that CMU is readily adsorbed by certain soil fractions (6). The thermodynamic properties of the adsorbed CMU-water system are such that when the water passes through the soil there would be an alternate adsorption-desorption process occurring. This should give rise to a "wave" of CMU concentration at various depths throughout the soil. This wave function should lag behind the solvent front akin to the phenomena encountered in chromatography.

The data presented herein bear out the foregoing hypothesis. It was found that there was a periodicity to the movement of the CMU through the soil columns which was a function of the amount of water added. It was found that the curves in Figure 2-a and Figure 2-b could be accurately portrayed by the following equation:

$$y = xe^{-c/x}$$

Where $y$ = depth in inches of primary wave peak
$x$ = number of "inches of rainfall"
$c$ = constant for a given soil at a specified moisture level
$e$ = base of the natural logarithm

The constant "c" was found to equal 2 inches for the sandy soil used in this study and equal to 2.5 inches for the clay loam used under the conditions of this experiment. Cognizance should be taken of the fact that diffusion and evaporation at the surface will bring about equilibration wherein the location of maximum concentration will not be sharply defined. This will result in a lower wave of broader base (3).

From these facts it is deduced that the distribution of CMU in the soil has a time dependency factor that involves the rate of percolation of water past a given point in the soil horizon and the interaction of restoring forces such as diffusion.

## SUMMARY AND CONCLUSIONS

This study was conducted in an effort to determine the effect of soil moisture, precipitation, and soil type on the movement of radioactive CMU in the soil.

The two different soil types, Chehalis sandy loam and Willamette clay loam, were separately mixed, sifted, and diluted to the predetermined moisture level of 15 per cent and 35 per cent on the sandy soil and 15 and 40 per cent in the clay soil. These soils were uniformly packed into 9 inch by 10 mm soft glass tubes, the radioactive CMU was placed on top of each column, and the water was added at a rate equivalent to 1, 3, or 9 inches of rainfall.

After 24 hours, the tubes were divided into segments, and the segments were analyzed for the radioactive carbon.

It was found that leaching of CMU increased with increasing water percolation. Leaching was greater in sandy soil than in a clay loam soil.

The distribution of the maximum was found to be described by an equation involving the amount of water passing a given point in the soil and a constant of the given soil considered.

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# Chemical Studies on *Halogeton glomeratus*[1]

W. B. Dye[2]

## Introduction

*Halogeton glomeratus*, a weed poisonous to animals, was first observed in the United States near Wells, Nevada, 1934–5 (2, 7). Dayton (4) has given a detailed historical sketch concerning its European-Asiatic habitats and species. The ecology of halogeton has been discussed by Cook and Stoddard (3), Fenley (5) and Tisdale and Zappettini (8). Very little is known in the English-speaking world about the present status of *Halogeton glomeratus* in Asia, where this species is reported to be endemic.

Burge[3] estimated that the 1954 infestation of Nevada is roughly more than two million acres, ranging from a few plants per acre to heavily infested areas. Figure 1 shows the distribution of halogeton in Nevada during the 1954 season. Other western states, particularly Idaho and Utah, report large increases in halogeton acreage.

That halogeton is toxic to animals, and that this toxicity is due to its high oxalate content was established by Fleming, Vawter and Miller in 1942 (6, 7, 9), following the loss of several hundred sheep in northeastern Nevada. Subsequently O'Harra and Dye[4] working with cattle found that only an aqueous extract of the plant would poison animals; the solid extracted portion was non-toxic. They further observed that if the aqueous extract were treated with an equivalent amount of calcium ion to precipitate the oxalate, the filtrate was non-toxic. Poisonous inorganic constituents were ruled out since a solution of the ash force-fed to cattle caused no toxic symptoms. The clinical symptoms from ingesting an amount of sodium oxalate equivalent to a lethal dose of halogeton appear to be identical. These results

Fig. 1. Halogeton infestation of Nevada, 1954. (Courtesy L. M. Burge).

[1]Studies conducted at the Agricultural Experiment Station, Max C. Fleischmann College of Agriculture, University of Nevada, Reno, Nevada.

[2]Agricultural Research Chemist, University of Nevada.

[3]Private communication from L. M. Burge, Supervising Inspector, Division of Plant Industry, Nevada State Department of Agriculture.

[4]O'Harra, J. F., and Dye, W. B., University of Nevada Agricultural Experiment Station. Unpublished.

indicate that only the salts of oxalic acid are responsible for the plant's toxicity.

## ANALYTICAL PROCEDURE

Considerable work was done by Miller,[5] in developing a method for the analysis of oxalate in halogeton. The following procedure has given excellent results:

### *Preparation of Sample*

The plants were clipped off at the surface of the ground and either air-dried or oven-dried at 90–100°C. If inorganic constituents were to be determined accurately, the sample was washed four times with petroleum naphtha before air drying, to remove the excessive dirt and sand which contaminated this desert plant. For routine oxalate determinations this precaution was usually not necessary. After extraction of the oxalate, the sample was treated to remove the pectic substances which interfere in the permanganate titration of the oxalate.

### *Water Soluble Oxalate*

A 2.000 gram sample was digested on a steam bath for one hour in a 250 ml volumetric flask containing 200 ml of distilled water. The flask was cooled, made to volume and filtered clear. For analysis two 50 ml aliquots were placed in 250 ml beakers. Ten ml 6 N sulfuric acid was added to each aliquot and evaporated to about one half original volume. This caused the separation of a flocculent precipitate, brown for mature and light green for immature samples. The solution was filtered and the residue washed 8–10 times with hot water. The residues were discarded.

### *Precipitation*

To the filtrate of 100–125 mls, 3–4 drops of methyl red indicator and then concentrated ammonium hydroxide were added until salmon pink color changed to faint yellow. The solution was heated on a steam bath to 90–100°C. Ten ml of 5 per cent calcium chloride solution was then added slowly with constant stirring. After 10 minutes 6 N ammonium hydroxide was added slowly with stirring, until faint yellow, then 20–25 drops in excess. The solution was set aside at least 4 hours (preferably overnight). The calcium oxalate was filtered on S and S 597 (or equivalent) filter paper and washed with water 6–8 times or until filtrate was free of calcium ion.

### *Titration*

The filter paper was left on the funnel, and the point of cone broken with a platinum wire. The precipitate was washed into the beaker in which the oxalate was precipitated, with several 5 cc volumes of hot sulfuric acid (1 + 4). The solution was diluted to 125 ml, heated to 90–100°C on a steam bath and titrated with 0.05 N $KMnO_4$. Finally the filter paper was added to the solution and titration completed.

[5]Miller, M. R., Research Chemist Emeritus, Agricultural Experiment Station, University of Nevada. Unpublished.

*Total (Acid Soluble) Oxalate*

A 2.000 g sample in 190 ml water + 10 ml 6 N HCl was digested and treated as above.

*Calculations*

$$\frac{\text{Per cent oxalate}}{\text{(Dry basis)}} = \frac{\text{ml } KMnO_4 \times 0.05 \times 45.02 \times 100}{1000 \times \text{dry wt sample} \times 50/250}$$

*Other Constituents*

Crude protein, fiber ash, ether extract, calcium, phosphorus, etc., were analyzed by A.O.A.C. methods (1).

## RESULTS

*Seasonal Variation of Oxalate Content*

During 1952–3 monthly range samples of halogeton were taken at the Knoll Creek Experiment Station near Contact, Nevada. After air drying the stem fraction was separated from the leaf-seed-sepal fraction. Each fraction was weighed and moisture content and water-soluble oxalate determined separately. The oxalate present in the top growth of the plant could then be calculated. Table 1 shows the results obtained and these are plotted with Miller's average 1942–6 results for comparison, as shown in Figure 2. The results indicate that:

(1) The highest weight per cent of the leaf-seed-sepal fraction occurred in September at the location of this experiment (about 5500 feet elevation).

(2) The highest concentration of oxalate in the top growth for the 1952–53 season occurred in February, 1953. Miller's results,[5] which are an average in the 1942–46 period, show a maximum concentration in November, and steadily decreasing concentration thereafter. This month coincides with the usual beginning of the winter storms in that area. There are two factors which can account for the decrease in oxalate concentration of the top growth. One factor is the decrease of the leaf-seed-sepal fraction which contains 5–6 times as much oxalate as the stems. This is probably the predominant factor, and is largely dependent on wind. A typical picture illustrating this factor is shown in Figure 3 by Fenley (5). A minor factor is leaching by rain or snow. The results for 1952–53 for the leaf-seed-sepal fraction do not show any appreciable leaching between November 15 and February 1, during which time only 1.35 inches of precipitation occurred. The gist of the matter is that the concentration of the oxalate, or the toxicity of the top growth of the plant, will be dependent on the local seasonal weather conditions, particularly any condition affecting the amount of leaves, seeds and sepals on the plant. It might also be pointed out that cattle and sheep eating leaves, seeds, and sepals which have accumulated in drifts on the ground will require a lesser weight of plant material for a lethal dose than if they ate the entire top growth, including stems.

*Table 1.* Seasonal variation of water soluble oxalate content in *Halogeton glomeratus*. (Knoll Creek, Nevada, 1953 season).

| Date sampled | Fraction of plant | Per cent dry weight of top growth | Per cent oxalate of fraction (dry basis) | Per cent* oxalate in top growth (dry basis) |
|---|---|---|---|---|
| June 15, 1952 | Leaf-seed-sepal | 65.05 | 22.23 | 15.9 |
| June 15, 1952 | Stem | 34.95 | 4.17 | |
| July 15, 1952 | Leaf-seed-sepal | 66.79 | 20.54 | 15.1 |
| July 15, 1952 | Stem | 33.21 | 4.20 | |
| Aug. 15, 1952 | Leaf-seed-sepal | 71.08 | 20.87 | 15.3 |
| Aug. 15, 1952 | Stem | 28.92 | 1.59 | |
| Sept. 15, 1952 | Leaf-seed-sepal | 81.16 | 19.53 | 16.5 |
| Sept. 15, 1952 | Stem | 18.84 | 3.67 | |
| Oct. 15, 1952 | Leaf-seed-sepal | 78.97 | 20.17 | 16.9 |
| Oct. 15, 1952 | Stem | 21.03 | 4.36 | |
| Nov. 1, 1952 | Leaf-seed-sepal | 73.92 | 26.50 | 20.8 |
| Nov. 1, 1952 | Stem | 26.08 | 4.73 | |
| Nov. 15, 1952 | Leaf-seed-sepal | 74.51 | 22.44 | 17.7 |
| Nov. 15, 1952 | Stem | 25.49 | 3.76 | |
| Dec. 1, 1952 | Leaf-seed-sepal | 71.56 | 28.10 | 21.6 |
| Dec. 1, 1952 | Stem | 28.44 | 5.06 | |
| Feb. 1, 1953 | Leaf-seed-sepal | 69.20 | 32.81 | 23.8 |
| Feb. 1, 1953 | Stem | 30.80 | 3.67 | |
| Feb. 15, 1953 | Leaf-seed-sepal | 66.97 | 31.31 | 22.2 |
| Feb. 15, 1953 | Stem | 33.03 | 3.86 | |
| Mar. 15, 1953 | Leaf-seed-sepal | 60.96 | 29.24 | 19.2 |
| Mar. 15, 1953 | Stem | 39.04 | 3.53 | |
| May 15, 1953 | Leaf-seed-sepal | 58.27 | 27.17 | 17.1 |
| May 15, 1953 | Stem | 41.73 | 3.02 | |

*Calculated.

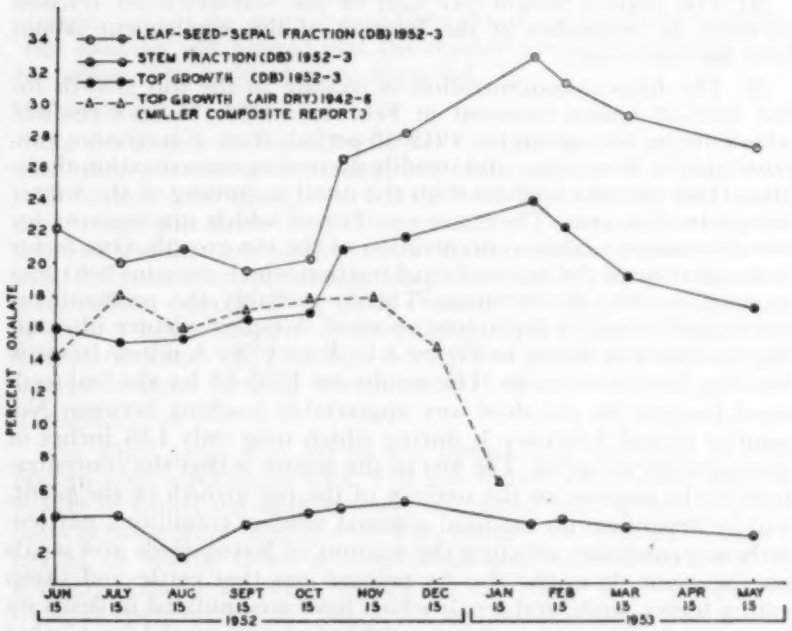


FIG. 2. Seasonal variation of oxalate content in *Halogeton glomeratus* in 1952-53. (Elko County, near Contact, Nevada).

Table 2 shows a proximate analysis of the leaf-seed-sepal fraction of a sample taken near Hazen, Nevada, in August, 1954. This was a fairly typical sample of halogeton taken at the time of maturity. Two interesting observations can be made from this analysis: (1) the ash content is extremely high even for a desert plant, and (2) the crude protein is higher than for an average alfalfa sample.

Table 3 shows the distribution of the major inorganic constituents of the leaf-seed-sepal fraction of Table 2. All of the mineral or inorganic constituents are derived from the soil; but oxalate, like the proteins, carbohydrates and fats must be synthesized by the halogeton plant.

*Table 2.* Proximate analysis of halogeton.

| Constituent | Leaf–seed–sepal* fraction per cent (dry basis) | Stem fraction per cent (dry basis) | Top growth per cent** (dry basis) |
|---|---|---|---|
| Crude protein | 17.98 | 7.83 | 16.36 |
| Ether extract | 5.51 | 0.83 | 4.76 |
| Crude fiber | 12.67 | 29.32 | 15.32 |
| Ash | 41.59 | 27.54 | 39.34 |
| Nitrogen free extract | 22.25 | 34.48 | 24.22 |
| Total | 100.00 | 100.00 | 100.00 |

*83.96 per cent by weight of top growth (dry basis).
**Calculated.

*Table 3.* Inorganic chemical and oxalate analysis of halogeton leaf—seed—sepal fraction.*

| Element | Per cent of fraction (dry basis) | Element | Per cent of fraction (dry basis) |
|---|---|---|---|
| Sodium as Na | 14.52 | Sulfur as $SO_4$ | 1.22 |
| Potassium as K | 0.19 | Phosphorus as P | 0.34 |
| Calcium as Ca | 0.73 | Silicon as $SiO_2$ | 1.49 |
| Magnesium as Mg | 0.20 | Oxalate ($H_2O$ soluble) as $H_2C_2O_4$ | 19.78 |
| Chlorine as Cl | 5.08 | Oxalate ($H^+$-soluble) as $H_2C_2O_4$ | 21.17 |

*All analyses, except for oxalate, made on an aqueous solution of the plant ash; oxalate analyses made on plant extracts.

It is very unfortunate that the plant's high oxalate content precludes its use as a stock feed. If the oxalate content could be reduced to a few per cent by some means (genetically perhaps), this plant would be highly desirable for desert ranges. It thrives on poor soil, low rainfall, no cultivation, and is green and succulent during the summer when other herbage is dry. It compares favorably with alfalfa with respect to protein content and it appears to have a higher percentage of phosphorus than the average alfalfa in Nevada. Halogeton's fire retardant property, due to its high ash content, is also a desirable characteristic.

## ACKNOWLEDGMENTS

The author desires to acknowledge the assistance of C. E. Fleming, Associate Director of the Agricultural Experiment Station under whose direction this work was done. He is also indebted to L. M.

FIG. 3. The loss of seed, sepals and leaves from halogeton, due to wind or storm. (Courtesy J. M. Fenley.)

Burge, Nevada State Department of Agriculture; M. R. Miller, Research Chemist Emeritus of the Nevada Experiment Station; J. M. Fenley of the Nevada Agricultural Extension Service; and Dr. W. C. Robocker of the U. S. Department of Agriculture, for their valuable aid in preparing this paper.

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# The Effect of 2,4–D and Several other Herbicides on Weeds and Soybeans when Applied as Post-emergence Sprays

F. W. SLIFE[1]

WITH the introduction of new chemicals for weed control, a tremendous effort has been expended toward finding a chemical weed control method for soybeans. Some of this research has been reported in the Research Reports and Proceedings of the North Central Weed Control Conference from 1946 to date. Much of the effort has been devoted to pre-emergence treatments on soybeans because of the apparent sensitivity of soybeans to foliage sprays of the present day herbicides.

The use of 2,4–dichlorophenoxyacetic acid (2,4–D) on soybeans as a post-emergence spray was investigated soon after 2,4–D became available for experimentation. In 1945, Stahler (6) summarizing work in the North Central states, classified soybeans as being extremely sensitive to 2,4–D sprays.

With the development of new formulations of 2,4–D and low volume spray equipment, several investigations were again carried out on the use of 2,4–D as a post-emergence spray on soybeans. Oyer and Lee (4) reported that when applications of 1/8, 1/4, 1/2, and 1 lb/A of 2,4–D acid in three different formulations of 2,4–D were made to Lincoln soybeans approximately 8 inches tall, injury was produced at all rates with all formulations. Injury symptoms were delayed maturity, lodging, and reduction in stand. Hanson and Freeman (3) studied the effect of 1/16, 1/8, 1/4, and 1/2 lb/A rates of 2,4–D amine and ester preparations and MCP amine on Wabash soybeans. These beans were harvested in the hay stage. The lower two rates of 2,4–D applied resulted in approximately 10 percent reduction in hay yield.

Smith (5) reported that he had used light rates of 2,4–D on soybeans on his farm for seven or eight years. He controlled cocklebur *(Xanthium spp.)* and horseweed *(Ambrosia trifida)* without seriously reducing the soybean yield. He stated that rates of application are very important to avoid decreasing yields.

Fribourg and Johnson (2) have recently reported the effects on the yield of 183 strains of soybeans from 2,4,5–T applications. Their work indicates that strains vary in their response to this chemical, and they suggest that plant breeders could use some of these strains in developing varieties that are more resistant to hormone sprays.

Even though soybeans were classified as being very sensitive to 2,4–D as early as 1945, there have been reports that farmers in the bottomland areas of the Ohio and Mississippi rivers use 2,4–D to control certain broadleaved weeds in soybeans. It has also been observed that although soybeans respond very rapidly to 2,4–D when it drifts on them from adjacent sprayed areas, they are seldom killed

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and usually recover. For these reasons, it was decided to study the effect of 2,4–D on soybeans more fully.

This study summarizes a 3-year investigation on the effect of various rates of 2,4–D on soybeans when applied as a post-emergence treatment. It also includes a study on the effect of various rates of 2,4–D on several of the common weed species found in soybeans and the effect of several different chemicals applied as a post-emergence spray to soybeans.

## MATERIALS AND METHODS

Field studies were carried out on the Agronomy Farm, Urbana, Illinois. In 1952, 1953, and 1954, Hawkeye soybeans were drilled in 40-inch rows at the rate of one bushel per acre. Plots were two rows wide and 35 feet long. There was a nontreated border row on each side of each plot. The design used was a randomized complete block with four replications. The beans were cultivated three times during the season to remove weeds so that the direct effect of 2,4–D on the soybeans could be studied more fully.

2,4–D treatments were made with an experimental plot sprayer similar to the push type machine described by Derscheid (1). The rates of triethenolamine 2,4–D used were 0, 1/16, 1/8, 1/4, and 1/2 pounds of acid per acre. Chemical and water diluent were applied at the rate of 20 gallons per acre to each plot.

In each of the three years the above rates of 2,4–D were applied to the soybeans at four different stages of growth. These growth stages were:

Stage I.....Soybeans 3 to 5 inches tall
Stage II.....Soybeans 7 to 9 inches tall
Stage III.....Soybeans 18 to 20 inches tall
Stage IV.....Soybeans 30 to 32 inches tall

In all three years data were taken on the effect of 2,4–D on the development, height, maturity, and yield of the soybean plants.

In 1953, 2,4–D amine was applied to seven species of broadleaved weeds common in soybeans. These weeds were: pigweed *(Amaranthus retroflexus)*, ragweed *(Ambrosia trifida)*, cocklebur *(Xanthium pennsylvanicum)*, morning glory *(Ipomoea purpurea)*, smartweed *(Polygonum pennsylvanicum)*, jimson *(Datura stramonium)*, and velvet leaf *(Abutilon theophrasti)*. Rates of 2,4–D used were 1/16, 1/8, 1/4, and 1/2 lb/A. These seven weed species were treated at three stages of growth approximating 2 to 4 leaves, 4 to 6 leaves, and 6 to 8 leaves. Notes were taken on the response of these weeds following treatment with 2,4–D.

In 1953 several other chemicals were compared with 2,4–D as a post-emergence spray on Hawkeye soybeans. The chemicals used were 2,4,5–trichlorophenoxyacetic acid (2,4,5–T), 2 methyl, 4–chlorophenoxyacetic acid (MCP), 3,4–dichlorophenoxyacetic acid (3,4–D), 4–chlorophenoxyacetic acid (4 Chloro), and 2,4–dichlorophenoxyacetic acid (2,4–D).

Rates of application used were 1/8 and 1/4 lb/A. The amine formulation of all chemicals was used in this test. Soybeans were treated at two stages of growth, 3 to 5 inches tall, and 7 to 9 inches tall. A randomized complete block design with four replications was used in this experiment, each plot being two rows wide and 35 feet long. Observations on the growth and development and yield of seed were taken from these plots.

## RESULTS AND DISCUSSION

Soybeans have considerable tolerance to 2,4–D in the early stages of growth but become more sensitive as they grow taller. The data on height maturity, lodging, seed germination, and yield presented in Table 1 and Figure 1 indicate that as much as 1/8 lb/A of 2,4–D acid in the amine form could be used on Hawkeye soybeans for weed control.

*Table 1.* Three-year average of the height at maturity, date of maturity, percent lodging, and germination of harvested seed of soybeans treated at four stages of growth with varying rates of 2,4–D.

| Stage of treatment | 2,4-D application lbs/A | Height at maturity in. | Maturity index[1] | Per cent of lodging | Germination of harvested seed—pct. |
|---|---|---|---|---|---|
| I 3"–5" tall | 0 | 40 | 0 | 0 | 86 |
| | 1/16 | 40 | 0 | 0 | 87 |
| | 1/8 | 38 | 0 | 0 | 86 |
| | 1/4 | 35 | +2 | 0 | 85 |
| | 1/2 | 32 | +4 | 15 | 70 |
| II 7"–9" tall | 0 | 40 | 0 | 0 | 88 |
| | 1/16 | 38 | 0 | 0 | 85 |
| | 1/8 | 35 | +2 | 0 | 86 |
| | 1/4 | 31 | +4 | 15 | 73 |
| | 1/2 | 28 | +5 | 55 | 51 |
| III 18"–20" tall | 0 | 40 | 0 | 0 | 88 |
| | 1/16 | 38 | 0 | 0 | 85 |
| | 1/8 | 35 | +2 | 0 | 86 |
| | 1/4 | 31 | +4 | 15 | 73 |
| | 1/2 | 28 | +5 | 55 | 51 |
| IV 30"–32" tall | 0 | 40 | 0 | 0 | 84 |
| | 1/16 | 38 | 0 | 0 | 86 |
| | 1/8 | 34 | +2 | 0 | 78 |
| | 1/4 | 30 | +4 | 20 | 64 |
| | 1/2 | 25 | +5 | 70 | 43 |

[1]Days earlier (–) or later (+) than the untreated check plots.

Soybeans responded very quickly to 2,4–D applied as a post-emergence spray (Figure 2). Within three hours the leaves of the plants began to fold or curl and the upper part of the plant began to bend. The maximum response occurred about 48 hours after spraying. After 48 hours, the soybeans began to recover at the lower rates of 2,4–D application, while the effect of the higher rates persisted for a longer period of time.

In all three years the 1/16 lb/A rate of 2,4–D was the least injurious to the soybeans at all stages of application and the injury from all rates was less at the first treatment and became more severe with each subsequent treatment.

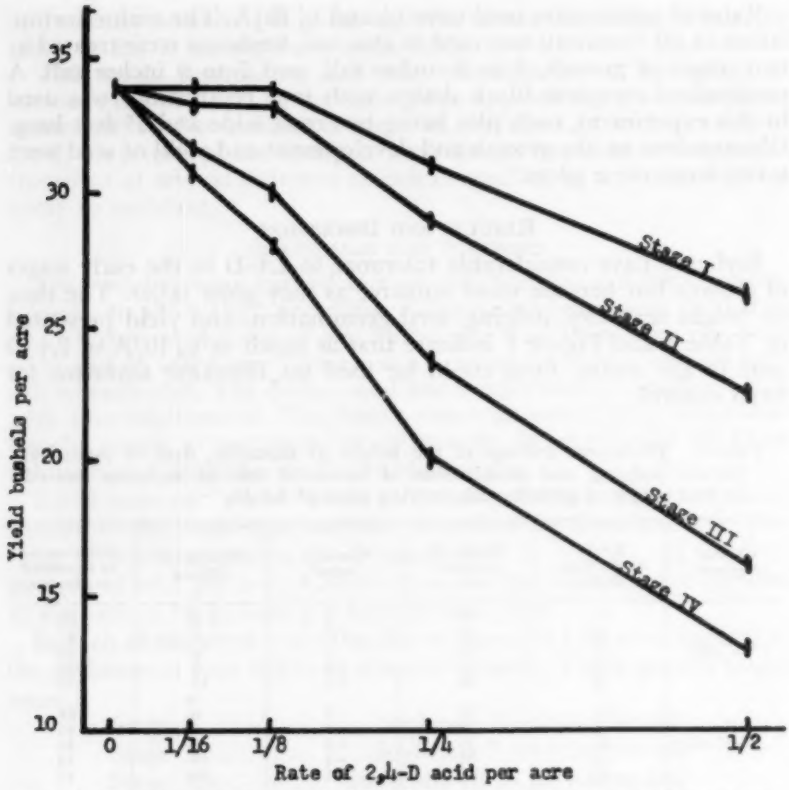


FIG. 1. The 3-year average seed yield of Hawkeye soybeans as influenced by various rates of 2,4-D applied at 4 stages of bean growth. Stage I—3 to 5 inches tall, Stage II—7 to 9 inches tall, Stage III—18 to 10 inches tall, Stage IV—30 to 32 inches tall.

Soybeans at the first treatment date seemed to have the ability to recover rapidly as compared to the later stages of growth. At the $^1/_{16}$ and $1/8$ lb/A rates, there was very little difference in height, maturity, lodging, or in seed germination as compared to the check (Table 1). The soybeans on the plots receiving the $1/4$ and $1/2$ lb/A rates were shorter, slightly delayed in maturity, and there was some lodging at the $1/2$ lb/A rate.

As the treatment dates progressed, the reduction in plant height, lodging, and reduction in yield became more severe with each treatment date (Table 1).

The $1/2$ lb/A rate caused severe lodging at the second, third, and fourth treatment dates. Since these plots were harvested with a small mower, all of the lodged plants were gathered and threshed. Under field conditions, a combine could not have picked up many of the lodged plants in the $1/2$ lb/A rate plots at the third- and fourth-treatment stages.

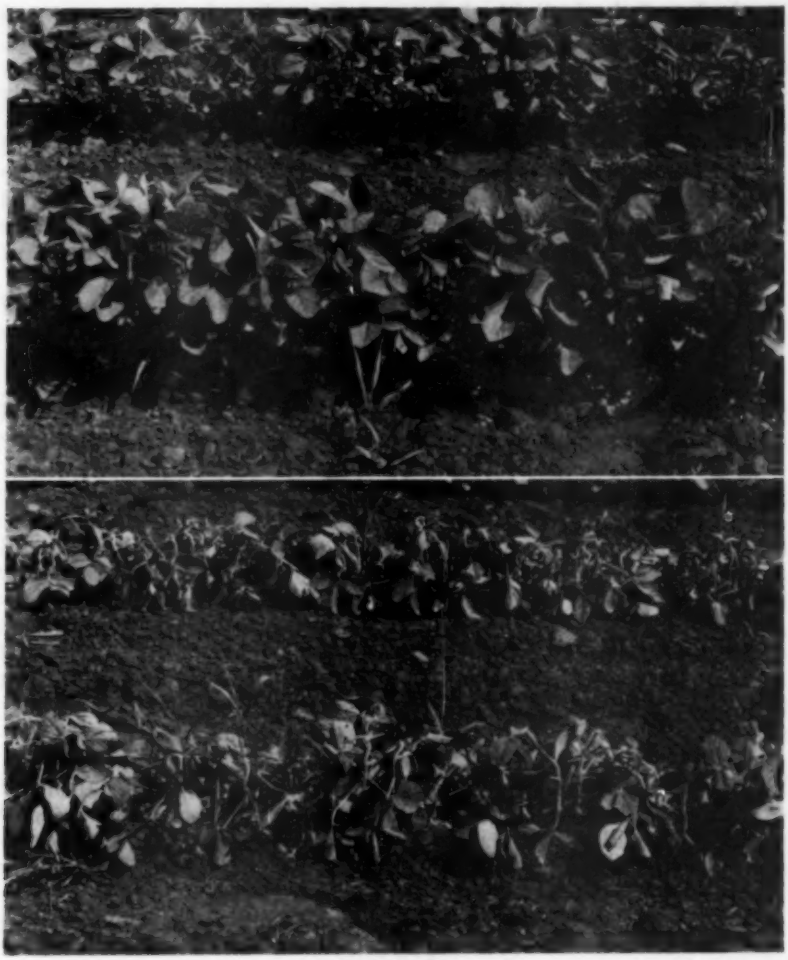

FIG. 2. The response of soybeans eight inches tall to 1/8 lb. of 2,4–D acid in the amine form. Pictures taken 48 hours after spraying. Upper picture untreated, lower picture treated.

Table 1 also gives the 3-year average of seed germination from the plots in this experiment. The seed germination of the untreated plot beans was not high due to abnormally dry weather during the three years of this test which reduced soybean germination below normal. Seed germination was affected at the 1/2 lb/A rate of 2,4–D at all treatment stages and at the 1/4 lb/A rate at stages III and IV.

Yield data are given in Figure 1. The curves represent the average yield for the three years for each rate of 2,4–D at each of the four treatment stages.

The individual data were analyzed by the analysis of variance

method and the individual degrees of freedom for the treatments were separated out so that more specific comparisons could be made. These comparisons indicate that the decreased yields brought about by increasing the rate of 2,4–D applications were less in the early treatment stages than in the later treatment stages.

Soybeans seem to have considerable tolerance to 2,4–D when small (Stage I), but yields are affected as the rates are increased. As the height of the beans increases, the 2,4–D has more of a detrimental effect on yield. The tolerance of small soybeans to 2,4–D indicates that it would be possible to use this chemical for the control of certain broadleaved weedy species.

Only one variety of soybeans has been studied in this series of experiments. It would be important to study the response of other common varieties to 2,4–D. Fribourg and Johnson (2) have already pointed out that strains of soybeans vary in tolerance to 2,4,5–T and, if common varieties are likely to vary as much to 2,4–D, they should be tested before this treatment is used in different areas of the soybean belt.

The nature of soybean tolerance to hormone sprays should also be studied. Close examination of sprayed plants showed that, due to the dense pubescence on the leaves and stems, the spray droplets did not contact the leaf surface directly. It would appear that the spray droplets were held from the leaf surface by the epidermal hairs. Soybean tolerance may be due to the fact that these hairs were less efficient in their adsorption of the chemical than the actual leaf surface. It also seems likely that there was less total surface contact with the chemical than there would be on plants that do not have the pubescence. Since soybean strains vary in the amount of pubescence they have, this point should be studied further.

The results of the test in 1953, where the amine formulations of 2,4,5–T, MCP, 3,4–D, 4 Chloro, and 2,4–D were applied at $\frac{1}{8}$ and $\frac{1}{4}$ lb/A of acid on soybeans 3 to 5 inches tall and soybeans from 7 to 9 inches tall, showed that there was little difference in response to the chemicals except that 4 Chloro caused less injury to the soybeans. 2,4,5–T, MCP, 3,4–D, and 2,4–D all produced similar bending and distortion of the soybean plants. There was no difference in the height of plants, amount of lodging, delay in maturity, yield, or germination of harvested beans from the plots treated with equal rates of 2,4,5–T, MCP, 3,4–D, and 2,4–D. The yield from the 4 Chloro treated plots were slightly higher than from the other treatments and there was no reduction in germination of the harvested seed.

In this test, 2,4,5–T, MCP, and 3,4–D caused injury similar to 2,4–D. Therefore there would be no particular reason for using these chemicals in preference to 2,4–D. All three of these chemicals are more expensive than 2,4–D and, as yet, have not been shown to be any better for control of most annual broadleaved weeds. Although 4 Chloro caused the least amount of injury and yield reduction, in general it is less toxic to annual broadleaf weeds than the other chemicals.

The results of using 2,4–D on seven common weeds frequently found in soybeans indicated that all of these weeds were affected more by the 2,4–D in the 2- to 4-leaf stage than in the 4- to 6-leaf or the 6- to 8-leaf stage. This test also clearly points out the wide degree of susceptibility among annual broadleaved weeds to 2,4–D. The $^1/_{16}$ lb/A rate did not kill any of the seven species in any of the three treatments, but it did reduce the growth of cocklebur, ragweed, and annual morning glory. 2,4–D at $1/8$ lb/A rate killed cocklebur, ragweed, morning glory, and pigweed at the 2- to 4-leaf stage and reduced the growth markedly at the other two stages. Jimson, smartweed, and velvet leaf were not killed at this rate, but were controlled well at the 2- to 4-leaf stage, and partially controlled at the last two stages of treatment.

The $1/4$ lb/A rate killed all species except velvet leaf and jimson at the 2- to 4-leaf stage, and it killed all species except smartweed, jimson, and velvet leaf at the later stages. Even though these species were not killed, they were controlled to such an extent that they were no real problem the rest of the season.

At the $1/2$ lb/A rate, all of the weeds were killed at the 2 to 4-leaf stage, and at the last two stages all weeds were killed except jimson, velvet leaf, and smartweed. In these latter three species, the stand was reduced over 60 percent and they were controlled satisfactorily.

The results indicate that the annual broadleaved weeds included in this test are most sensitive to 2,4–D in the early stages of growth. Rates as low as $1/8$ lb/A seem sufficient to eliminate or effectively control such species as ragweed, cocklebur, morning glory, and pigweed. Jimson, smartweed, and velvet leaf are not usually eliminated at this rate, but are often controlled to such an extent that they may not compete seriously for the essentials of growth.

More investigations are needed on the nature of the tolerance of some of the broadleaved weeds to 2,4–D. If the variation in resistance is caused by lack of chemical penetration, then perhaps the resistance of the weeds could be altered by changing the components of the spray, such as the wetting agent.

## SUMMARY

A 3-year study on the effect of various rates of 2,4–D on soybeans indicated that soybeans have considerable tolerance to this chemical in the early stages of growth but become more sensitive as they grow older. Seed yields were not affected by $^1/_{16}$ or $1/8$ lb/A of 2,4–D acid in the amine form when applied to soybeans 3 to 5 inches tall and 7 to 9 inches tall. The yields were affected slightly at the same rates when soybeans were sprayed at 18 to 20 inches tall. The $1/4$ and $1/2$ lb/A rates of 2,4–D affected yields at all stages but were less severe when applied at 3 to 5 inches tall.

Seven weed species common in soybeans were sprayed with $^1/_{16}$, $1/8$, $1/4$, and $1/2$ lb/A of 2,4–D acid as the amine form at three different growth stages. These stages were 2 to 4 leaves, 4 to 6 leaves, and 6 to 8 leaves. All species were more sensitive at the first stage of growth

and became progressively more tolerant to the chemicals with each advanced stage of treatment. Cocklebur, ragweed, morning glory, and pigweed were killed with as low as 1/8 lb/A of 2,4–D acid in the 2 to 4-leaf stage. Jimson, smartweed, and velvet leaf were affected but very few plants died.

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## News and Notes

Dr. Anson R. Cook has recently been employed by the Oklahoma A. & M. Botany Dept. to work on weed and brush control in that state. Mr. Cook received his Ph.D. degree in 1953 from the University of Michigan. He worked at the Hawaii Agricultural Experiment Station in Honolulu in 1953 and 1954.

J. K. Leasure has accepted a position with the agricultural chemical research staff of the Dow Chemical Co. and is now located at Midland, Michigan. Mr. Leasure was formerly engaged in weed work at the University of Tennessee.

After receiving his Ph.D. degree from the Dept. of Horticulture of the University of Wisconsin, C. A. Shadbolt accepted a position with the University of California at Davis. Mr. Shadbolt will be working on the physiology of vegetable crops and with weed control in these crops.

T. W. Tibbitts has returned to the Dept. of Horticulture, University of Wisconsin to work on physiology of tobacco and to do some weed control in this crop. Mr. Tibbitts received his Ph.D. degree from the University of Wisconsin in 1953 and then served two years in the armed forces.

O. E. Rud, formerly research assistant with the Dept. of Agronomy and Plant Genetics, Univ. of Minnesota, has accepted a position as Research Instructor with the Agronomy Department, North Carolina State College. Mr. Rud will carry on the weed control research work in cotton, soybeans, peanuts and forage crops, replacing R. P. Upchurch who was called to active duty in the Air Force.

Robert Frans, who recently received his Ph.D. degree from Iowa State College, has joined the staff of the Agronomy Dept. of the University of Arkansas. He will be engaged in weed control research at the latter institution.

R. J. Smith, Jr. was appointed Agronomist, Weed Investigations Section, ARS, USDA, Stuttgart, Arkansas, after receiving his Ph.D. degree from the University of Illinois. Mr. Smith is working on weed problems in rice in cooperation with the Arkansas Agricultural Experiment Station.

Laurence C. Walker recently accepted a position with the School of Forestry, University of Georgia. One of Mr. Walker's primary responsibilities will be research on the elimination of undesirable hardwoods from piedmont pine stands with chemical and cultural procedures.

Donald L. Huss has been named Instructor in the Dept. of Range and Forestry, Texas A. & M. College at College Station. Mr. Huss will devote part-time on brush control research with emphasis on control and management of juniper and ecological surveys of brush population in South Texas.

W. C. Normand was appointed Assistant Plant Physiologist at the Mississippi Agricultural Experiment Station, State College, Mississippi. He recently received his Ph.D. degree at Louisiana State University where his research was concerned with studies of some physi-

ological responses of cotton to herbicides. Mr. Normand will devote full-time to laboratory and greenhouse studies on basic physiological problems pertaining to weed control.

John T. Holstun, Jr. returned to the Weed Investigations Section, ARS, USDA, Stoneville, Mississippi after taking leave of absence to do graduate study at Iowa State College. His doctoral research problem concerned the phytotoxicity and reactions of 2,2–dichloropropionic acid in soils. Mr. Holstun will do full-time research on both basic and applied weed control problems with special emphasis on cotton.

Garlyn O. Hoffman has been appointed Extension Range Specialist in the Texas A. & M. College System at College Station. Mr. Hoffman and A. H. Walker, Extension Range Specialist, have conducted an extensive series of demonstrations throughout Texas of brush control by means of basal trunk sprays and injections, soil sterilants, and soil injections.

O. Hale Fletchall has accepted a position with the University of Missouri in which he will be spending one-half time teaching and one-half time on research in weed control. He was formerly a cooperative agent of the University of Missouri and the Field Crops Research Branch, USDA.

S. Clark Martin, Range Conservationist with the U.S. Forest Service, has been transferred to Laramie, Wyoming. He was formerly active in brush control research work in Missouri.

A. R. Midgley resumed his duties as Chairman of the Agonomy Dept. of the University of Vermont after a two-years leave of absence. Mr. Midgley spent the past two years in Bolivia under the point IV program where he helped initiate the use of many of the newer weed control practises.

Frank Salisbury has accepted a position with the Colorado Agricultural Experiment Station and will specialize in studies on weed physiology. He replaces Roger Blouck who recently resigned.

A. R. Hamson, who has been engaged in extension work in weed control in canning and processing crops in New York State, has resigned and accepted a position with the Utah State Agricultural College, Logan, Utah. Mr. Hamson will continue part-time work in the field of weed control.

Stanford Fertig has taken a two year's leave of absence from the Dept. of Agronomy, Cornell University to do weed control work in the Philippines under the exchange professor program.

W. B. McHenry has been appointed by the Extension Service of the University of California to assist W. A. Harvey with the weed control program in that state. One of Mr. McHenry's main duties will be putting out test plots with farm advisors.

The eighth annual meeting of the California Weed Conference and the fifteenth Western Weed Control Conference will be held jointly in Sacramento, California on February 15 and 16, 1956 and in Davis on February 17.

The ninth meeting of the Southern Weed Conference is to be held at the Jung Hotel in New Orleans, Louisiana on January 16, 17, and 18, 1956.

State weed workers in Florida have organized themselves into a group known as "The Weed Control Workers of the University of Florida". Purposes of the organization include making annual tours to observe weed control experiments as well as to keep all workers fully informed of progress in various phases of the program.

The Third International Conference on Plant Growth Substances was held from July 17 to 22, 1955 at Wye College, Ashford, Kent, England. Sessions on "The Natural Auxins", "Chemical Structure and Biological Activity", "Metabolism of Auxins", and "Mode of Action of Auxins" were included in the program. The proceedings of this conference will be published in a volume to be entitled "The Chemistry and Mode of Action of Plant Growth Substances".

A six-weeks Agricultural Pilot Training Course, sponsored by the Texas Aerial Applicators Association, U.S. Civil Aeronautics Administration and The Texas A. & M. College, was given at Texas A. & M. College during November and December 1955, under the leadership of Fred E. Weick. Purpose of the course was to train commercial pilots in the aerial application of agricultural materials. Considerable attention was given to the problems of weed and brush control by aerial applications of herbicides.

Noel S. Hanson reports that a Hawaii Weed Conference has been organized with sections on the islands of Hawaii, Kauai, Maui and Oahu. The officers of the conference are; President, Harold Hall, Pioneer Mill Co., Lohaina, Vice-President, Charles Willett, Olaa Sugar Co., Olaa, and Sec.-Treasurer, Noel Hanson, Experiment Station, Hawaii Sugar Planters Assn., Honolulu.

## SUSTAINING MEMBERS

American Chemical Paint Company, Ambler, Pennsylvania
American Smelting and Refining Company, South Plainfield, New Jersey
Carbide & Carbon Chemicals Company, New York, New York
Chipman Chemical Company, Inc., Bound Brook, New Jersey
G.L.F. Soil Building Service, Ithaca, New York
The Dow Chemical Company, Midland, Michigan
National Aluminate Corp., Chicago, Illinois
Oberdorfer Foundries, Inc., Syracuse, New York
Penn Line Service Inc., Scottdale, Pennsylvania
Pittsburgh Coke & Chemical Company, Pittsburgh, Pennsylvania
Spraying Systems Company, Bellwood, Illinois
Standard Agricultural Chemicals, Inc., Hoboken, New Jersey
Swift & Company (Plant Food. Div.), Chicago, Illinois

# BIBLIOGRAPHY OF WEED INVESTIGATIONS
## for July, August, September, October, November, and December, 1954

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This bibliography of current literature on weeds and their control is issued as a service to those engaged in weed research in the United States and other countries. In issuing this bibliography the Section of Weed Investigations does not assume responsibility for the subject matter cited.

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